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CLINICAL AND EXPERIMENTAL

A STUDY OF THE LACTOBACILLI WITH SPECIAL REFERENCE TO THE BACILLUS ACIDOPHILUS*

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THIS investigation was originally undertaken to determine the potency of the commercial preparations of Bacillus acidophilus which are offered for sale on the American market. It was felt that the claims of the manufacturers, as described in the printed literature accompanying their viable preparations, were somewhat exaggerated and a scientific investigation of these statements would be of value to both the medical profession and the public in general. Preliminary examination of the various strains of bacteria isolated from numerous commercial preparations indicated that through proper cultivation any member of the lactobacilli group will assume the morphologic and cultural characteristics of any other desired bacillus of this group. This investigation was then extended to include a study of the lactobacilli group as a whole. It was found that any of the lactic organisms obtained from the gastrointestinal tract could, by employing the proper food supply, be changed into an organism having the morphologic and cultural characteristics of any desired member of the lactobacilli group. The change took place through a definite cycle and depended upon the nature of the food on which the organism was cultured, the P_H value of the media, and the incubation temperature.

As early as 1908 Rodella[1] claimed that the Boas-Oppler bacillus was identical with B. acidophilus and B. bifidus of Tissier. Heinemann and Hefferan[2] employing organisms obtained from sour and aromatic foodstuffs, cattle foods, milk and fermented milk purchased on the open market, manured and unmanured soil, normal saliva, normal gastric juice, gastric juice in the absence of

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hydrochloric acid, and human, bovine, and horse feces, concluded that B. Boas-Oppler, B. panis fermentati, Streptobacillus lebensis, Leptothrix buccalus, B. acidophilus, and B. bifidus were all identical with B. bulgaricus. The only objection that could be raised to the above investigation was the failure of the authors to employ a sufficiently large variety of cultural criteria. More and Rahe[3] described three varieties of B. acidophilus which they termed A., B., and C. Variety C they reported identical with B. bulgaricus. Variety B was a streptobacillus exhibiting the characteristics of B. acidophilus, Moro. Variety C was a long bacillus or streptobacillus which was similar to B. acidophilus, Finkelstein. Kulp and Rettger[4] concluded that the difference between B. bulgaricus and B. acidophilus constituted sufficient evidence for their classification as separate species. Kopeloff,[5] however, suggested that the gram-positive rods be called Lactobacillus bulgaricus and the Lactobacillus acidophilus be placed in the same species, with L. acidophilus as the central type. L. bulgaricus may then be considered a variant of this type, the variation being due to long culturing in milk, and its chief differential characteristic being its loss of ability to develop in the intestines, even when supplied as a milk culture.

A. I. Kendall[6] showed that the chemical action of bacteria depends in a great measure upon the medium in which it exists. Giuseppe Solarino[7] demonstrated the presence of a gram-negative phase in the development of gram-positive bacteria. Hadley[8] and Hauduroy[31] reported that bacteria have a stage in their development in which they become too small to be recognized by microscopic examination. In this form they were observed to pass through porous filters and were for a time non-cultivatable by ordinary methods. They may be made to develop into normal forms and in some cases weeks and months were required to affect this reversion. These various phenomena were observed in the present investigation and form the basis for the development of the cycle through which the lactobacilli pass.

Metschnikoff[9] claimed to have implanted B. bulgaricus in the human intestine but later investigations of Kopeloff,[5] Kulp and Rettger,[4] Kulp,[11] Rettger and Cheplin,[12] Clendenning,[13] Cruickshank,[14] and others[26, 27, 28] showed that this was not the case but B. acidophilus could be implanted. Cruickshank and Berry[15] were undecided as to whether actual implantation took place or whether the organism merely survived the exposure to acid. Hitzrot and Lynch[16] found that in combination with lactose, beneficial results were obtained in B. acidophilus therapy but "Implantation was obviously not achieved." James[17] (1927) stated that implantation was impracticable from the use of any commercial preparations examined by him at that time. H. V. Hughens[18] described the installation of living cultures of B. acidophilus directly into the colon and observed only an immediate temporary change of flora. This was the only recorded experimental procedure employing direct inoculation. In the other cases, the organism was subjected to the salivary and gastric fluids, thus introducing conditions which would allow a possible change in the morphology and growth characteristics. R. P. Smith[19] found that a change of flora over to the aciduric type did not influence the growth of the typhoid and paratyphoid organisms but it did suppress B. coli. Our results indicated that in the case of the lactobacilli, implantation could not occur with respect to a specific member of this group if the

conditions were favorable for the development of any other member and were foreign to the original bacillus. It is true they may survive as long as the artificial media containing the specific food substances for their characteristic development is provided, but by preference they will revert to the type that is characteristic of the surrounding media. Kendall has shown that many strains of bacteria produce nontoxic end products in carbohydrate media, and when grown aerobically, but under adverse conditions, they can become anaerobic and utilize the amino acids for food with the consequent production of toxic substances. Thus B. diphtheria in a carbohydrate medium grew aerobically and produced nontoxic lactic acid, while in a media containing proteinous products it grew anaerobically and produced the highly active toxic substance, diphtheria toxin.

Our results indicated that if B. acidophilus were introduced into the small intestine, only a relatively small number of the bacilli would become implanted by reverting over to an organism having the characteristics of another member of the lactobacilli group whose natural habitat is the adult human intestine. This inversion may cause the harmless B. acidophilus to become a toxic producing organism.

EXPERIMENTAL

The strains of the lactobacilli employed in this investigation were obtained from six different sources and purported to be Lactobacillus acidophilus. The source and fermentation characteristics of these strains, before purification, are recorded in Table I.

TABLE I

STRAINS	** 1	** 2	** 3	4	5	6
Source	Commercial paste	Commercial chocolate covered cubes	Commercial cubes	Special laboratory strain used for commercial preparation	Special laboratory strain used for commercial preparation	Isolated from infant feces
Sugars						
Raffinose	++	++	++	++	+	++
Sucrose	++++	++++	++++	++++	++	+++
Lactose	++++	++++	++++	++++	++	++++
Maltose	+++	+++	+++	+++	+++	+++
Inulin				++++	+++	
Glucose	+++	++++	++++	+++	+++	+++
*Mannitol	none	none	none	none	none	none
*Sorbital				none	none	
Dextrin				none	none	none
Xylose	++	++	++	++	++	++
Salicin				none	none	
Dulcite	none	none	none			

*Filtered.
**Labeled to contain millions of viable bacteria.

The six strains of Table I were purified for use in this investigation in the following manner: Strains 4 and 5 were plated out in tomato whey agar. A characteristic colony selected and transferred to tomato whey broth. The bacillus was again plated out on tomato whey agar, fished, and transferred to

tubes of tomato whey and whey agar. Strain 6 was obtained by the Cruickshank and Berry technic and plated on tomato whey agar. A characteristic colony was selected and the organism isolated by means of the modified Barber's technic. It was then grown in tomato whey, transferred to tomato whey agar, fished and inoculated into tomato whey broth, and again fished and transferred to whey agar. Strains 1, 2, and 3 were obtained by trituration and emulsification in sterile saline, plating on tomato whey agar, fishing the large and small (pin head

TABLE II
CHARACTERISTICS OF THE PURIFIED STRAINS

STRAIN	1	2	3	4	5	6
Morphology						
Size	2μ dia.	2μ dia.	2μ dia.	1 × 5μ	1 × 5μ	1 × 6μ
Form	circular	circular	circular	rods	rods	rods
Arrangement	singly and chains	singly and chains	singly and chains	end to end	end to end	end to end
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos.	pos.	pos.
Remarks	three elements in chain					
Cultural Characteristics						
Growth	surface	surface	surface	surface and deep	surface and deep	surface and deep
Milk	coagulation	coagulation	coagulation	coagulation and separation of whey in 172 hr.		
Litmus milk	acid	acid	acid	acid in 72 hr.		
Whey	growth	growth	growth	growth and sediment appearing at the end of 72 hr.		
Russel's media				growth in butt growth in liquid		
Tomato agar	small, grayish-white colonies			small, smooth, raised colonies 0.7 to 1.0μ dia.		
Lactose broth	growth; singly, pairs, and in chains of three					
Acidity	acid reaction			strong acid production		
Fermentation						
Raffinose				++	+	++
Sucrose	++++	++++	++++	+++	+++	+++
Lactose	++++	++++	++++	++++	++++	++++
Maltose	++++	++++	++++	+++	++++	+++
Glucose	++++	++++	++++	+++	+++	+++
Galactose	+++	+++	+++	++++	++++	++++
Dextrin				none	none	none
Mannitol				none	none	none
Gas Production	none	none	none	none	none	none

and pin point), grayish-white, translucent colonies to tomato whey, diluting 1:100 with sterile distilled water, and replating in whey agar. Characteristic colonies, of both the pin head and pin point types, were then selected and the organism isolated by the Barber-Chamber technic.[24] The inoculation was made in whey, transferred to tomato whey, and finally grown in whey agar.

The morphology and cultural characteristics were then determined for the various strains and are recorded in Table II. The organisms from the two

TABLE III

DEVELOPMENT OF THE PURIFIED STRAINS OF LACTOBACILLI ON SELECTIVE MEDIA

STRAIN	4	5	6	4	5	6
MEDIA	TOMATO DIGEST (DEXTROSE)			TOMATO DIGEST (LACTOSE)		
MEDIA ROTATION	1	1	1	2	2	2
Morphology						
Size	1 × 8μ	1 × 7μ	1 × 8.5μ	0.8 × 5μ	0.8 × 5μ	0.8 × 4μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	pairs and chains	pairs and chains	pairs and chains	singly and chains	singly and chains	singly and chains
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos.	pos.	pos.
Remarks	A few take the gram stain poorly			Often chains of 8 elements are found		
Cultural Characteristics						
Growth	best anaerobic			best anaerobic		
Milk	acid with coagulation and no digestion of clot			clotted in 72 hr. with whey slowly separating		
Litmus milk	acid with coagulation			acid with coagulation		
Butter milk	slight growth			no growth at end of 72 hr.		
Tomato milk	gram negative forms			no growth		
Whey	no growth			no growth		
Kulp's tomato	no growth			no growth		
Tomato digest	gram pos. and neg. rods			growth		
S and W media	growth			growth		
S and W digest	growth			growth		
S and W sucrose	very slight growth			slight growth		
Whey agar	no growth			fair growth		
Nutrient agar	no growth			slight growth		
Broth	very slight growth			very slight growth		
Lactose broth	poor growth			poor growth		
Glucose broth	abundant growth			abundant growth when acidified with acetic acid		
Acidity	about 1 per cent as acetic			about 10 per cent in 72 hr. as acetic (very high)		
Fermentation						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	+			++		
Sucrose	+			++		
Dextrin				+		
Mannitol	none			+		
Gas Formation	none			in all tubes		
Incubation Temp.	37° C.			37° C.		
Strain	1	2	3	1	2	3
Media Rotation	8	8	8	9	9	9

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	SMITH AND WALLGREN (GALACTOSE)			SMITH AND WALLGREN DIGEST (SUCROSE)		
MEDIA ROTATION	3	3	3	4	4	4
Morphology						
Size	0.6 × 5μ	0.6 × 4.5μ	0.6 × 5μ	0.3 × 2-4μ	0.3 × 3-5μ	0.4 × 1.5-5μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly and end to end	singly and end to end	singly and end to end	singly bifurcations	singly bifurcations	singly bifurcations
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos. and neg.	pos. and neg.	pos. and neg.
Remarks	Ends frequently tapering			Showed true branching		
Cultural Characteristics						
Growth	both aerobic and anaerobic			anaerobic		
Milk	coagulates after 48 hr.			acid, generally coagulates after 96 hr.		
Litmus milk	acid with soft coagulum in about 48 hr.			acid, coagulation very slow in occurring		
Butter milk	slight growth in about 72 hr.			slight growth in 96 hr.		
Tomato milk	slight growth in 48 hr.			slight growth in 72 hr.		
Whey	some gram negative forms			no growth		
Kulp's tomato	no growth			no growth		
Tomato digest	growth, gram pos. & neg. rods			involution forms		
S and W media	growth			gram pos. and neg. rods		
S and W digest	single rods, good growth			growth		
S and W sucrose	involution forms			abundant growth		
Whey agar	pin-point, grayish colonies translucent, fimbriate margins			growth		
Broth	slight turbidity, grayish sediment			no turbidity		
Lactose broth	growth			slight growth		
Glucose broth	gram pos. and neg. forms			abundant growth		
Acidity	quite low; 5.5 per cent in 5 days			acid		
Fermentation						
Dextrose	++++			++++		
Galactose	++++			++		
Lactose	++++			+		
Maltose	++++			++++		
Sucrose	++++			++++		
Dextrin	none			++		
Mannitol	none			+++		
Gas Formation	none			none		
Incubation Temp.	38° C.			38° C.		
Strains	1	2	3	1	2	3
Media Rotation	10	10	10	11	11	11

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	SMITH AND WALLGREN DIGEST (LACTOSE)			ALPHA WHEY BROTH (LACTOSE)		
MEDIA ROTATION	5	5	5	6	6	6
Morphology						
Size	0.8 × 2-6μ	0.8 × 2-6μ	0.8 × 3-5μ	0.8 × 4-7μ	0.8 × 4-7μ	0.8 × 4-7μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	end to end only	end to end only	end to end only	singly and long chains	singly and long chains	singly and long chains
Motility	none	none	none	none	none	none
Gram staining	pos. and neg.	pos. and neg.	pos. and neg.	pos.	pos.	pos.
Remarks	No chains			Fluorescent points at the poles		
Cultural Characteristics						
Growth	aerobic and anaerobic			best growth anaerobic but grows aerobic		
Milk	excellent growth but no coagulation			acid with no clotting		
Litmus milk	acid; no coagulation			acid; no separation of whey but soft creamy curd deposited in time		
Butter milk	involution forms			growth		
Tomato milk	involution forms			some gram neg. forms		
Whey	involution forms both gram pos. and neg.			growth		
Kulp's tomato	no growth			no growth		
Tomato digest	no growth			involution forms		
S and W media	growth			growth		
S and W digest	growth			growth		
S and W sucrose	small rods round on both ends			gram pos. and neg. rods		
Whey agar	slight growth			small, flat, circular colonies		
Glucose agar	growth			growth		
Broth	no growth in 24 hr.			slight growth		
Lactose broth	excellent growth			turbid, growth		
Glucose broth	abundant growth					
Acidity	0.2 per cent in 24 hr. as acetic			high		
Fermentation						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	++			+++		
Mannitol						
Gas Formation	none			none		
Incubation Temp.	37° C.			37° C.		
Strains	1	2	3	1	2	3
Media Rotation	12	12	12	13	13	13

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	THETA WHEY BROTH (GLUCOSE AGAR)			BUTTER MILK PLUS GLUCOSE TO THETA WHEY BROTH		
MEDIA ROTATION	7	7	7	8	8	8
Morphology						
Size	0.3 × 1.5μ	0.3 × 3μ	0.3 × 2.5μ	0.3 × 1-2μ	0.3 × 1-2μ	0.3 × 1-2μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly	singly	singly	singly and in chains	singly and in chains	singly and in chains
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos.	pos.	pos.
Remarks				Chains of 2 to 6 elements some of the rods showed both gram pos. and neg. segments		
Cultural Characteristics						
Growth	facultative anaerobes; best growth anaerobic			aerobic and anaerobic		
Milk	coagulates in from 6 to 12 days; soft curd; no retraction			coagulates in 7 hr. at 45° C.		
Litmus milk	acid			acid		
Butter milk	slight growth			growth		
Tomato milk	gram pos. and neg. forms			growth		
Whey	scanty growth			involution forms		
Kulp's tomato	involution forms			involution forms		
Tomato digest	involution forms			involution forms		
S and W media	involution forms			involution forms		
S and W digest	gram neg. and some gram pos.			gram pos. and neg.		
S and W sucrose	gram neg. and pos.			gram pos. and neg.		
Whey agar	very scant growth					
Whey glucose	growth			growth		
Glucose agar	growth			growth		
Broth	involution forms			involution forms		
Lactose broth	growth			3 element chains		
Glucose broth	good growth			good growth		
Acidity	very slight			slight		
Fermentation						
Dextrose	~~++++~~			~~++++~~		
Lactose	~~++++~~			~~++++~~		
Maltose	~~+++~~			~~+++~~		
Sucrose	~~+++~~					
Gas Formation	none			none		
Incubation Temp.	40° C.			40° C.		
Strains	1	2	3	1	2	3
Media Rotation	14	14	14	15	15	15

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	GALACTOSE WHEY			GLUCOSE WHEY		
MEDIA ROTATION	9	9	9	10	10	10
Morphology						
Size	1-1.5μ	1-1.5μ	1-1.5μ	1-1.5μ	1-1.5μ	1-1.5μ
Form	circular	circular	circular	circular	circular	circular
Arrangement	pairs	pairs	pairs	singly	singly	singly
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos.	pos.	pos.
Remarks	In lactose whey pairs and single forms occur			True nitrifier		
Cultural Characteristics						
Growth	facultative anaerobes; best growth anaerobic			facultative anaerobes		
Milk	rapid coagulation by fermentation			coagulation by acid (lactic) and not fermentation		
Litmus milk	slowly changes acid			acid		
Butter milk	growth			growth		
	butter milk plus galactose gives a gram pos. pair surrounded by gram neg. sheath					
Tomato milk	growth			growth		
Whey	growth			growth		
Kulp's tomato	growth			growth		
Tomato digest	growth			growth		
Whey lactose	both single and pairs			circular forms arrange themselves in chains of 3; gram neg. sheath surrounds some of these triads; length 3-4μ		
S and W media	sheathed pairs			growth		
S and W digest	growth			growth		
S and W sucrose	growth			growth		
Broth	growth			growth		
Lactose broth	single and pairs			single; pairs, and triads		
Glucose broth	growth			growth		
Acidity	slight acid reaction			acid reaction		
Fermentation						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	+++			++++		
Sucrose				++++		
Dextrin				++		
Mannitol				+++		
Gas Formation	none in 48 hr.			none in 48 hr.		
Incubation Temp.	37° C.			37° C.		
Strains	1	2	3	1	2	3
Media Rotation	16	16	16	17	17	17

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	LACTOSE WHEY TO MILK			LACTOSE WHEY		
MEDIA ROTATION	11	11	11	12	12	12
Morphology						
Size	0.5 × 1-2μ	0.5 × 1-2μ	0.5 × 2-3μ	0.8 × 3-6μ	0.8 × 3-6μ	0.8 × 3-6μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly and chains	singly and chains	singly and chains	singly and chains	singly and chains	singly and chains
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos.	pos.	pos.
Remarks						
Cultural Characteristics						
Growth	facultative anaerobes			facultative anaerobes		
Milk	no coagulation but good growth			no coagulation but good growth		
Litmus milk	unchanged			acid but no coagulation		
Butter milk	slight growth			growth		
Tomato milk	growth			no growth		
Whey	involution forms			no growth		
Kulp's tomato	no growth			no growth		
Tomato digest	growth			no growth		
Whey lactose	reverts back to circular forms			abundant growth		
S and W media	growth			growth		
S and W digest	no growth			no growth		
S and W sucrose	involution forms			involution forms		
Broth	growth			growth, very slight		
Lactose broth	growth			growth		
Glucose broth	growth			no growth		
Whey agar	growth			no growth		
Lactose agar	growth			small, grayish, viscid colonies		
Acidity	acid reaction			acid reaction		
Fermentation						
Dextrose	++++			++		
Galactose	++++			+++		
Lactose	++++			++++		
Maltose	++++			none		
Sucrose	++++			none		
Dextrin	++++					
Mannitol	++++			none		
Gas Formation	none			in dextrose and lactose		
Incubation Temp.	37° C.			40° C.		
Strains	1	2	3	1	2	3
Media Rotation	18 or 1	18 or 1	18 or 1	2	2	2

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	GALACTOSE WHEY			DELTA WHEY BROTH (MALTOSE)		
MEDIA ROTATION	13	13	13	14	14	14
Morphology						
Size	1 × 3-4μ	1 × 3-4μ	1 × 3-4μ	1 × 4-6μ	1 × 4-6μ	1 × 4-6μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly	singly	singly	single and chains	single and chains	single and chains
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos. and neg.	pos. and neg.	pos. and neg.
Remarks				chains of 2 to 3 elements the rods showed both gram pos. and neg. segments		
Cultural Characteristics						
Growth	facultative anaerobes good aerobic growths			facultative anaerobes good aerobic growths		
Milk	coagulates into a firm clot; good growth; no peptonization			coagulation		
Litmus milk	acid			acid with coagulation		
Butter milk	growth			growth		
Tomato milk	growth			growth		
Whey	growth			growth		
Kulp's tomato	involution forms			involution forms		
Tomato digest	no growth			growth		
Whey lactose	growth			coccoid shaped forms		
S and W medium	growth			involution forms		
S and W digest	slight growth			slight growth		
S and W sucrose	no growth			growth		
Broth				slight turbidity		
Lactose broth	slight growth			slight growth		
Glucose broth	growth			slight growth		
Whey agar	small, transparent colonies					
Acidity	4 per cent in dextrose medium			2-5 per cent in dextrose medium		
Fermentation						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	++++			++++		
Sucrose	++++			++++		
Mannitol				++++		
Gas Formation	none			none		
Incubation Temp.	37° C. and 35° C.			37°-34° C.		
Strains	1	2	3	1	2	3
Media Rotation	3	3	3	4	4	4

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	WHEY			KULP'S TOMATO WHEY		
MEDIA ROTATION	15	15	15	16	16	16
Morphology						
Size	1 × 3-16μ	1 × 3-16μ	1 × 3-16μ	2 × 4-20μ	2 × 4-20μ	2 × 4-20μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	chains	chains	chains	chains	chains	chains
Motility	none	none	none	none	none	none
Gram staining	pos.	pos.	pos.	pos. and neg.	pos. and neg.	pos. and neg.
Remarks	Chains with no true branching. Often over 200μ in length			Chains with 2 to 10 elements. The organisms did not attack casein		
Cultural Characteristics						
Growth	aerobic			facultative anaerobes, best growth anaerobic		
Milk	coagulates with a firm clot and little serum			acid and coagulation in from 10 to 15 hr.		
Litmus milk	acid with curd			acid with soft, creamy curd in 24 hr.		
Butter milk	abundant growth			abundant growth		
Tomato milk	growth			involution forms		
Whey	abundant growth			turbid, grayish-white sediment		
Kulp's tomato	growth			abundant growth		
Tomato digest	involution forms			growth		
S and W medium	involution forms			growth		
S and W digest	involution forms			rods divide into gram pos. and gram neg.		
S and W sucrose	no growth			revert to coccoid shape		
Whey agar	small, white colonies about 1 mm. in dia.			circular to irregular, grayish-white, filamentous colonies		
Acidity	1.5-2.5 per cent			2.7-4 per cent in milk		
Fermentation						
Glucose	+++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	+++			++++		
Mannitol				++++		
Gas Formation	none			none		
Incubation Temp.	37° C.			40° C.		

Strains	1	2	3	1	2	3
Media Rotation	5	5	5	6	6	6

TABLE III (Concluded)

STRAINS	4	5	6	4	5	6
MEDIA	TOMATO NUTRIENT BROTH			TOMATO DIGEST DEXTROSE		
MEDIA ROTATION	17	17	17	18 or 1	18 or 1	18 or 1
Morphology						
Size	0.5 × 7μ	0.5 × 7μ	0.5 × 7μ	1 × 8.5μ	1 × 8.5μ	1 × 8.5μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	single and chains	single and chains	single and chains	pairs and chains	pairs and chains	pairs and chains
Motility	none	none	none	none	none	none
Gram staining	pos. and neg.	pos. and neg.	pos. and neg.	pos.	pos.	pos.
Remarks	In liquid media a space appears between elements, rods often appear in parallel			Some stain poorly		
Cultural Characteristics						
Growth	facultative anaerobes			facultative anaerobes		
Milk	coagulates in 6 hr. and produces 0.3 per cent acid 37° C.			acid with coagulation and no digestion of clot		
Litmus milk	acid and no digestion of clot			acid with coagulation		
Butter milk	slight growth			slight growth		
Whey	slight growth			no growth		
Kulp's tomato	gram pos. and neg. rods			no growth		
Tomato digest	growth			gram neg. and pos. forms		
S and W media	growth			growth		
S and W digest	involution forms			growth		
S and W sucrose	involution forms			slight growth		
Whey agar	slight growth			no growth		
Nutrient agar	no growth			no growth		
Lactose agar	growth					
Lactose broth	growth			slight growth		
Glucose broth	growth			growth		
Acidity	0.3 per cent in 24 hr. as lactic			1 per cent in milk in 24 hr.		
Fermentation						
Glucose	++++			++++		
Galactose				++++		
Lactose	++++			++++		
Maltose	+++			++		
Sucrose				-		
Mannitol				none		
Gas Formation	none			none		
Incubation Temp.	37° C.			37° C.		
Strains	1	2	3	1	2	3
Media Rotation	7	7	7	8	8	8

characteristic colonies (2 to 4 mm. dia. and 0.7 to 1 mm. dia.) were identical in morphology but differed culturally in so far as the larger colonies were surface growths and aerobic, while the smaller colonies were deep growers and anaerobic.

The various purified strains were then successively grown on a series of different media. The morphology of the organism was checked every twenty-four hours by staining with Kopeloff's modified gram stain[25] and examining under the microscope. At various intervals, depending upon a change in morphology of the organism, the cultural characteristics were determined. The results are recorded in Table III. Strains 4, 5, and 6 were rotated successively on tomato digest containing dextrose, tomato digest containing lactose, S and W medium containing galactose, predigested S and W medium containing sucrose, predigested S and W medium containing lactose, lactose whey broth, glucose whey broth, buttermilk fortified with glucose, glucose whey broth, galactose whey, glucose whey, lactose whey, milk, lactose whey, galactose whey, maltose whey broth, whey, Kulp's tomato whey, tomato nutrient broth, and tomato dextrose digest medium. Strains 1, 2, and 3 were transferred to whey, buttermilk fortified with galactose, lactose whey, milk, glucose whey, and then rotated as in strains 4, 5, and 6, starting with glucose whey. The method of preparation of these various culture media is described in a subsequent paper.

The number of times an organism was transferred to the same media, the length of time of incubation, and the point at which a new medium was employed, depended upon the morphologic characteristics of the organism when stained and viewed under the microscope. A considerably longer time was required to transform the circular into the rod shape than the reverse.

The morphologic changes and cultural characteristics are recorded in Table III. A review of the literature on the morphology and cultural characteristics of the more definitely established members of the lactobacilli group[20, 21, 22, 23] describe Lactobacillus lactisacidi (Leichmann) as a rod shaped organism varying from 0.8 to 1.2 micron in length and 0.5 micron in breadth. They appear singly and in chains. The ends of the bacillus are square and they are gram-positive. Optimum temperature 40° C. Their natural habitat is milk. They are microaerophilic and grow in milk and broth, and form soft, grayish, transparent filiform growths on agar slants. Litmus milk is unchanged. They do not form indol nor reduce nitrates. Acid is produced. Acid fermentation was reported in dextrose, maltose, fructose, lactose, and dextrin without the formation of gas. Arbinose and trehalose were not fermented.

Lactobacillus helveticum (lactobacillus casei) are described as rod shaped organisms, 0.7 to 0.9 micron in breadth and 2 to 6 microns in length. They appear singly and in chains, nonmotile, and gram-positive. Optimum temperature 40 to 42° C. Their natural habitat is sour milk and cheese. They are microaerophilic, do not coagulate milk, and show no growth in broth, whey gelatin, whey agar, nor gelatin. Deep growth occurs in lactose agar, as well as in small, grayish, and viscid colonies. Litmus milk becomes acid without coagulation. They do not form indol nor reduce nitrates. Acid fermentation with the production of gas occurred in lactose and dextrose. Maltose and mannitol were not fermented.

Kornchen bacillus of Leurssen and Kuhn, are described as rod shaped organ-

isms, nonmotile, and gram-positive. They stain in Neisser and alkaline methylene blue. Optimum temperature 37 to 45° C. Their natural habitat is buttermilk. Good growth occurs in carbohydrate media (milk plus carbohydrates) with the coagulation of the milk into a firm clot at a temperature of 37° C. There is no evident peptonization. Indol is not formed, nor nitrates reduced. Acid fermentation occurs in carbohydrate medium without the production of gas.

Lactobacillus pabuliacidi (Bergey) are described as rod shaped organisms occurring singly and in long chains. They are nonmotile and gram-positive. Optimum temperature 34 to 40° C. Their natural habitat is beet mash and cheese. They are aerobic, coagulate milk, and produce a slight turbidity in broth. A slightly arborescent growth occurs in gelatin stab, and small, transparent colonies on agar. Four per cent acidity is produced in dextrose. Acid fermentation, without the production of gas, occurs in lactose, sucrose, maltose, mannitol, and glycerol. Raffinose is not fermented. Litmus milk becomes acid without coagulation.

Bacterium mazun (Weigmann, Gruber, and Huss) are described as gram-positive rods, 1 to 1.1 microns in breadth and 2.7 to 21 microns in length. They form chains with no true branching, and are nonmotile. The ends of the rods are swollen, and cells with true branching are reported in whey. Involution forms occur in the shape of rods 210 microns in length. They stain with carbolfuchsin, and their optimum temperature is 37° C. They are aerobic, coagulate milk with a firm clot and little serum, and grow abundantly in whey. Small, white colonies about 1 mm. in dia. occur in whey agar. They produce acid.

Lactobacillus bulgarious are described as rod shaped organisms, 1 to 2 microns in length (Bergey), 2 to 20 microns in length (Ford), and forming chains. They are gram-positive in young cultures and gram-negative in old ones. They are nonmotile, stain with all ordinary stains, and form chains of 2 to 10 elements in length. They are facultative anaerobes. They grow rapidly in milk, coagulation occurring in from ten to twenty-four hours at 37° C. and an acidity of from 2.7 to 3.7 per cent is produced, i. e., 32.5 gm. lactic acid per liter of milk in ten hours while other members produce about 10 grams per liter in the same length of time. Litmus milk becomes acid in twenty-four hours with the production of a soft, creamy curd. They produce a turbidity in whey with a grayish white sediment, and circular to irregular, grayish-white, filamentous colonies on whey agar. The agar colonies are small, thick, lenticular, umbilicated and 0.1 mm. in diameter. Acid fermentation, without the production of gas, occurs in dextrose, lactose, galactose, fructose, and mannitol. Rhamnose, dulcite, and sorbite are not fermented. This organism does not attack casein, has its natural habitat in yoghurt and buttermilk, and its optimum temperature is 45° C.

Streptobacillus lebensis (Rist and Khoury) are described as rod shaped organisms, nonmotile, 0.5 micron in breadth, and 6 to 8 microns in length. They occur singly and in chains up to 18 microns in length. They have been reported found in parallel bundles, and in liquid media a space appears between the elements of the chain. They are gram-positive in young cultures, but the dead organisms are gram-negative. Optimum temperature 37 to 40° C. Their natural habitat is fermented milk. They grow aerobic and anaerobically and coagulate

milk in six hours at 37° C. with the production of 0.26 per cent acid, calculated as lactic. A true rennin ferment is produced with no digestion of the clot. A slight growth is obtained in whey and better growths on glucose and lactose agar. Gas is not produced on fermentation. They stain with carbolfuchsin.

Lactobacillus Boas-Oppleri (Boas and Oppler) are described as rod shaped organisms occurring in pairs and chains, nonmotile, and gram-positive. Accordin to Ford these rods are 1 micron in breadth and from 2 to 10 microns in length while Bergey finds them 6 to 8 microns in length. Red metachromatic granules are obtained with methylene blue. Optimum temperature 40° C. Their natural habitat is the gastric contents, especially gastric carcinoma. Although microaerophilic, they grow best anaerobically. Best growths are obtained on acid media, broths plus glucose or beer wort, and media containing gastric cancer extract. Litmus milk is turned acid with coagulation, and small, gray, granular colonies appear on milk agar. One per cent acidity is produced in milk. Nitrates are not reduced. Acid fermentation without the production of gas occurs in dextrose and lactose. Mannitol is not fermented.

Lactobacillus acidophil-aerogenes (Torrey and Rahe) are described as rod shaped organisms, gram-positive, occurring singly and in chains up to 40 microns in length with occasional branching or Y forms. The rods are 0.8 micron in breadth and from 1.5 to 11 microns in length (Bergey gives 5 microns as the maximum length). They are nonmotile and stain with Loeffler's alkaline methylene blue. Optimum temperature 37° C. Their natural habitat is the intestinal tract. They can grow aerobically and anaerobically, and clot milk in seventy-two hours with the partial separation of whey. Acid coagulation occurs in litmus milk. Growth occurs on gelatin and agar but best on glucose oleate agar or 5 per cent acetic acid glucose. Nitrates are not reduced. Ten per cent acidity is produced in three days. Acid fermentation occurs in dextrose, lactose, sucrose, and raffinose, with the production of gas. Arabinose is not fermented.

Lactobacillus acidophilus are described as slender, gram-positive rods occurring singly and in short chains usually of 2 to 3 elements. They are from 4 to 5 microns in length and the ends of the rods are found to be frequently tapered. They are nonmotile and the metachromatic granules are stained by Neisser's method. Optimum temperature 37 to 45° C. Their natural habitat is the human infant intestine. They grow both aerobically and anaerobically. Milk is generally reported to be coagulated after forty-eight hours and litmus milk becomes acid with the production of a soft coagulum in the same length of time. They produce a slight turbidity in broth with a gray sediment. Gray, fimbriate colonies slowly develop on gelatin and very scanty growth occurs in the gelatin stab. The colonies on agar are thin, irregular, and translucent with fimbriate margins, and the slant shows a filiform, smooth, and slimy growth. Good growth occurs on glucose agar. Indol is not formed. This organism produces a low acidity, 5.5 per cent in five days in dextrose broth. Acid fermentation without the formation of gas occurs in dextrose, lactose, maltose, raffinose, and sucrose. Mannitol and dextrin are not fermented.

Lactobacillus bifidus communis (Tissier) are described as rod shaped organisms, 0.3 to 0.6 micron in breadth and 1.5 to 6 microns in length. They occur singly, showing bifurcations and true branching. The young cultures are gram-

positive, while the old cultures and the dead organisms are gram-negative. They are stained with basic anilin dyes. Optimum temperature 37° C. Their natural habitat is the intestine. They are strict anaerobes and grow abundantly in glucose broth. Acid coagulation of milk takes place in time, and litmus milk may or may not be coagulated. Poor growth occurs in gelatin. The colonies on glucose agar are deep seated and have an average diameter of 2 mm. with a maximum of 4 mm. Acid fermentation occurs in dextrose, lactose, maltose, sucrose, raffinose, and mannitol.

Lactobacillus lebensis (Rist and Khoury) are described as rod shaped organisms, 2 to 6 microns in length and arranged end to end without the formation of chains. The young cultures are gram-positive, while the old cultures and dead organisms are gram-negative. They are nonmotile and appear granular (similar to the tubercle bacilli) in whey. Their natural habitat is milk. They grow both aerobically and anaerobically and produce about 0.2 per cent acid (as lactic) in twenty-four hours. Excellent growth occurs in milk without coagulation, and litmus milk is turned acid. Good growth occurs in lactose or glucose broth, whey, glucose or lactose agar, and lactose whey.

Lactobacillus caucasicus (Kern; Beijerinck) are described as nonmotile, gram-positive, rods, 0.8 micron in breadth and 3.2 to 8.0 microns in length. They occur singly and in long chains and have glistening points at the poles. Optimum temperature 37 to 40° C. They grow both aerobically and anaerobically; the growth often being delayed two to three weeks. Milk becomes acid without clotting. Litmus milk develops 1.2 to 1.6 per cent acidity (as lactic) and a soft creamy curd. Lactose broth becomes turbid, and a growth occurs in both whey agar and whey gelatin. Small, flat, grayish, circular, undulated surface colonies, and deep, fimbriate colonies occur in agar. The growth on agar slants is scanty, while a grayish growth occurs in agar stabs. Indol is not formed, nor are nitrates reduced. Acid fermentation without gas production occurs in dextrose and lactose. Mannitol is not fermented.

Bacillus exilis (Tissier) are described as nonmotile, gram-positive rod shaped organisms, whose natural habitat is the stools of infants. They stain with dilute fuchsin, gentian violet, and thionin. Their optimum temperature lies between 37 and 40° C. They are facultative anaerobes and do not grow on acid media. They coagulate milk in eight to ten days with a soft curd and no retraction. Litmus milk is turned acid. A deep growth occurs in glucose agar at 20 to 37° C., and they produce no gas on fermentation.

Streptobacillus C. (Grigoroff) are described as nonmotile, gram-positive, rod shaped organisms. Their natural habitat is buttermilk. Optimum temperature 37 to 45° C. They are facultative anaerobes, coagulate milk in six hours at 45° C., and produce acid in litmus milk. They produce buttermilk at 37° C. in from twelve to twenty-four hours. They are true denitrifiers and do not form indol. Acid fermentation occurs in lactose, as well as in lactose broth.

Diplococcus lebensis (Rist and Khoury) are described as nonmotile, gram-positive, circular organisms, which always occur in pairs. These organisms take the usual stains and do not form spores. Growth occurs in glucose broth, whey, and lactose agar. A rapid coagulation occurs in milk, and when it is kept alkaline or neutral with sodium carbonate, a rennin-like ferment is produced.

Micrococcus B (Grigoroff) are described as nonmotile, gram-positive, circular organisms, which always occur singly. Their natural habitat is buttermilk. They grow both aerobically and anaerobically. Buttermilk is produced in less than twenty-four hours at 37° C. Milk is coagulated by the production of lactic acid and not by fermentation. Good growth occurs in glucose broth. These organisms are true nitrifiers and give a positive nitroseindol reaction. They are acid producing organisms. Acid fermentation occurs in fructose, rhamnose, maltose, saccharose, mannite, and glycerin. Dulcite and sorbite are not fermented.

LACTOBACILLI CYCLE

On comparison of the morphologic and cultural characteristics of Table III with those of the members of the lactobacilli group described in literature, a striking similarity between the various bacilli was observed. When Table III is arranged in the form of a cycle, Table IV is obtained. A critical study of Table IV brought out this peculiarity, that the cycle naturally divides itself into two

TABLE IV

LACTOBACILLUS CYCLE FROM PURIFIED STRAINS

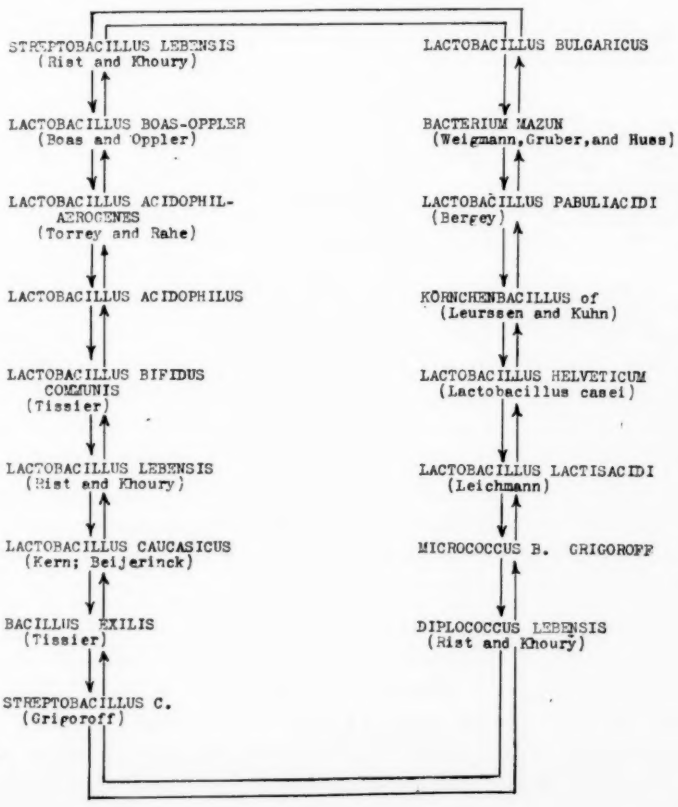
parts. The left-hand, or anaerobic part, in which the organisms were difficult to culture, required a specialized media based on the chemical and physical constitution of the contents of the intestine at different life periods, and grew best under anaerobic conditions at body temperature. The right-hand part consisted chiefly of facultative anaerobes cultured on milk and whey medium, and grew best under aerobic conditions. This is not a clear cut division, since some organisms, which are located at the top and bottom of the cycle illustrated in Table IV, grew equally well aerobically and anaerobically. The medium, in this case, giving the best results were of a combined nature.

Upon derivation of a cycle from such lactobacilli organisms as were found to be sufficiently described in literature, and arranging them according to the locations indicated in Table IV, Table V was obtained. This table showed that if we were to start with a pure culture of the Bacillus acidophilus, or in fact any other lactobacilli, we could obtain by a process of successive selective culturing any other desired member of this group. At least an organism having the morphologic and cultural characteristics of the desired bacillus could be obtained. These changes were regulated by the food supply (nature of the medium), the P_H value of the medium, and the temperature of incubation.

TABLE V

LACTOBACILLI CYCLE

ACCORDING TO THE NOMENCLATURE FOUND IN LITERATURE



THE NATURE OF THE MORPHOLOGIC CHANGE IN THE BACILLI

Two processes are involved, one of synthesis and the other of a destructive or degenerative nature. In the latter case the changes in the bacillus occurred quickly and readily, and ultimately terminated in a coccoid shaped end product. The process of synthesis required much skill in the rotation of medium, its selection, and the time of incubation. Starting with a chain, we observed a gram-negative section appearing between the elements of which it was composed. A separation then occurred at these junctions with no reduction in the length of the component rods. Gram-negative and gram-positive segments then appeared in the rods. Usually the gram-negative section was observed between two gram-positive components. In some cases the rod showed a division in the middle, half staining gram-positively and half gram-negatively. Division into gram-positive and gram-negative rods now occurred. The gram-negative rods

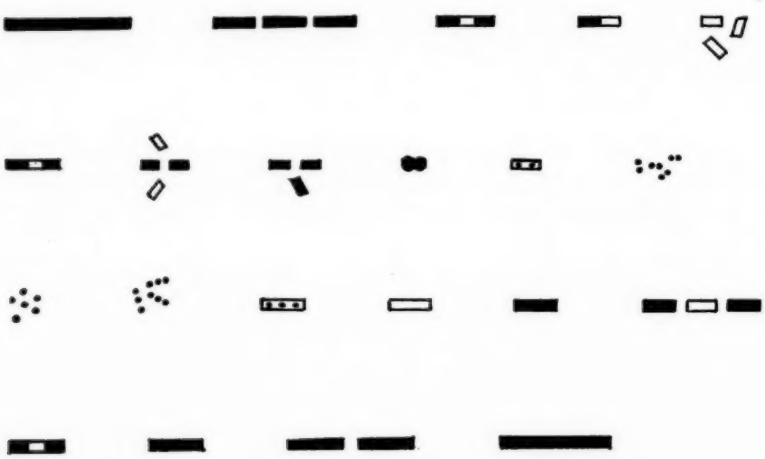


Fig. 1.—Involution and synthesis of the lactobacilli.

next changed over into gram-positive rods of the same size. These smaller rods again split as described above. When a length of from 1.5 to 2 microns was obtained, the rod slightly bulged out at a short distance from both ends, and stained gram-positively. These bulges developed into two circular elements contained at either end of the rod. On staining, the circular portion became gram-positive, and the rest of the rod, gram-negative. A few circular forms were observed connected by a restriction through their centers, the whole staining gram-positively, but this occured only in rare cases. Separation next occurred into circular pairs which took a gram-positive stain. The pairs were then observed to break up. The circular forms next arranged themselves into short chains generally consisting of three elements and stained gram-positively. A fusion then occurred, which on staining, showed three gram-positive circles contained within a gram-negative rod. The gram-positive circles then faded out leaving a gram-negative rod, which then changed over into a gram-positive rod. We were unable to determine at this point whether all the gram-negative rods changed over into gram-positive rods and then a specialized number changed back again

into gram-negative forms or only a part of them changed over into gram-positive rods. In every case, however, mixtures of gram-positive and gram-negative rods were obtained. The next step consisted in the fusion of the gram-negative and gram-positive rods into a single rod consisting of a gram-negative central portion and gram-positive ends. Three of the original rods entered into this fusion. This rod then changed over into a gram-positive rod of approximately the same length as the rods which entered into its synthesis. The size of the rod was then increased by the fusion of two gram-positive rods lying end to end, although, in some cases, fusion into a single rod occurred from chains of four

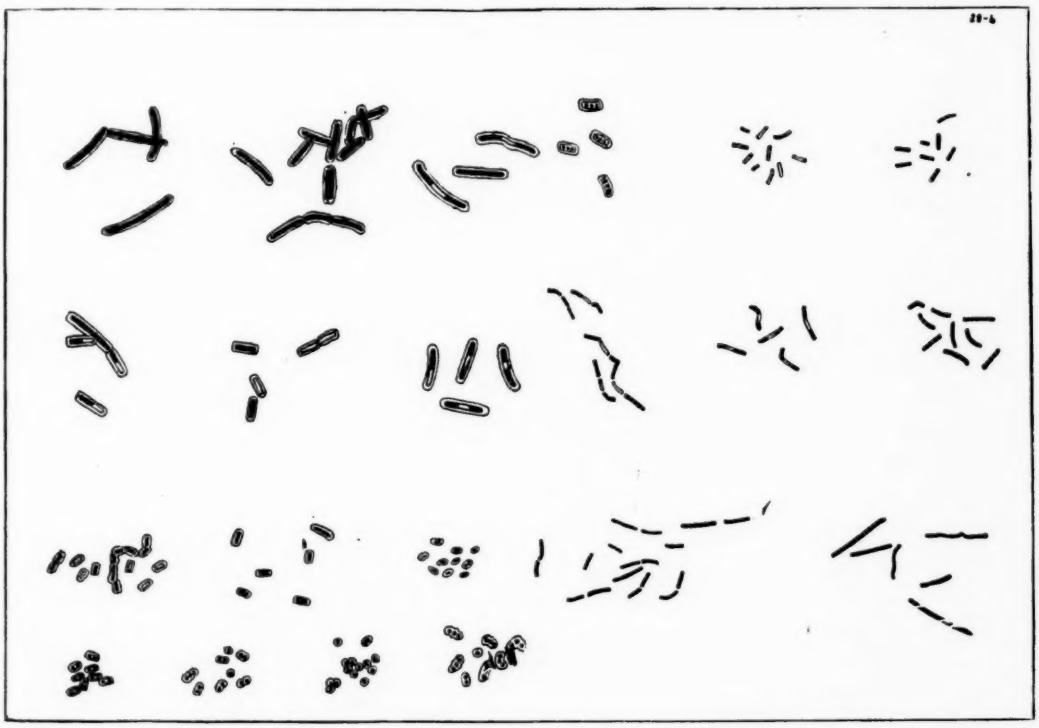


Fig. 2.—Morphologic changes in the culture of the lactobacilli. Camera lucida drawing 1650X.

gram-positive rods. This process is diagrammatically illustrated in Fig. 1. The scale of the diagram is exaggerated so as to bring out the details of structure. Since microphotographs did not bring out the gram-negative elements nor the capsulated forms, camera lucida drawings of the process were made and are reproduced in Fig. 2.

DISCUSSION

The results obtained in this laboratory indicate that any member of the lactobacilli group may be changed into, or caused to assume, the morphology and cultural characteristics of any other bacillus in this group through a successive culturing on selective media. The incubation period varied from a twenty-four-hour culture, in the case of the reduction in size of the rod, to one

hundred and ten hours. The higher figures were always characteristic of the synthesis of the rod and particularly from the circular forms. This process of synthesis is in agreement with the combined observations of Hadley, Solarino, Kendall, and Hauduroy.

The impracticability of the implantation of the Bacillus acidophilus now becomes apparent since it would revert over to a type acclimated to the surrounding media. This would be particularly true when treatment with the artificial media ceases, i. e., supplementary treatment with lactose, agar bran, paraffin, mucilaginous products obtained from seeds, etc. To continue the supplementary treatment required by acidophilus therapy over a long period of time would certainly produce a pathologic condition of the intermediate metabolism of the digestive system (including the enzymic and hormonic systems). To discontinue this supplementary treatment would soon result in a reversion of the introduced lactobacillus to a type further down the cycle, whose natural habitat is the normal adult intestine and is capable of utilizing the amino acids for food. Whether or not they digest the amino acids by deaminization, decarboxylation, or both, is at the present time uncertain, but under pathologic conditions (chronic constipation, etc.) they most certainly will aid in the production of toxic substances. We found that the Bacillus acidophilus grew best on an artificial media approximating as closely as possible the conditions found in the intestines when food passes through the duodenum of a breast-fed infant. After the infant is weaned, the intestinal flora changes rapidly and putrefactive types appear. It is our belief that the Bacillus acidophilus does not disappear from the adult intestine but will be found in relatively the same numbers in a form resembling the coccus or the diplococcus, and will have the cultural characteristics of that organism. Since the intestinal tract of a newborn infant is sterile, as well as the intestinal tract of numerous polar animals, and since it has been demonstrated that chicks and flys grown under sterile conditions function normally, we believe that the presence of bacteria of any description in the human intestinal tract is unnecessary and to add them is certainly unwise. For no matter what type of nonpathogenic organism may be added, they will revert to an organism capable of producing toxins, or interfering with the normal functioning of the body, according to its food supply.

SUMMARY

1. The morphology and cultural characteristics of the Bacillus acidophilus depends upon the nature of the food supply, the P_H value of the medium, and the presence of small quantities of inorganic metals, such as zinc, copper, iron, etc.

2. Any member of the lactobacilli group may be converted into, or caused to assume, the morphologic and cultural characteristics of any other member.

3. The members of the lactobacilli group may be arranged in a cycle according to their morphology and cultural characteristics.

4. Any member of this cycle in assuming the morphology and cultural characteristics of any other member passes successively through the characterization of all intervening members in the cycle.

5. The cycle is capable of division into two natural parts, one composed of members whose usual habitat is intestinal, and the other contains the nonintestinal members.

6. In the case of adverse conditions, if the organism survives, most of the nonintestinal and practically all of the intestinal forms assume the coccoid shape.

7. The change in morphology extends over a considerable period of time, even under the most favorable conditions. This is particularly true in the synthesis of the rod shaped bacilli.

8. The rods pass through a definite process in their synthesis and splitting, in which an interplay of gram-negative and gram-positive forms occur.

REFERENCES

1. Rodella, A.: Magencarcinom und Milchsaurebacillen (Boas-Opplerscher bacillus, Bacillus gastrophilus und Bacterium gastrophilum Lehmann-Neumann, Bacillus acidophilus und Bac. bifidus communis), Zentralbl. f. Bakteriol **47**: 445-466, 1908.
2. Heinemann, P. G., and Hefferan, M.: A Study of Bacillus bulgaricus, J. Infect. Dis. **6**: 304-318, 1909.
3. Rahe, A. H.: An Investigation Into the Fermentative Activities of the Aciduric Bacteria, J. Infect. Dis. **15**: 141-150, 1914.
4. Kulp, W. L., and Rettger, L. F.: Comparative Study of Lactobacillus acidophilus and Lactobacillus bulgaricus, J. Bacteriol **9**: 357-394, 1924.
5. Kopeloff, N.: Lacto-Bacillus Acidophilus, 1926.
6. Kendall, A. I.: Bacteria and the Chemist, J. Indust. Eng. Chem. **15**: 1001-1002, 1923.
7. Solarino, G.: Ricerche sulla Gram-resistenza dei germi; Su una fase Gram-negativa nello sviluppo di germi Gram-positivi, Boll. d. Soc. ital. di biol. sper. **5**: 98-100, 1930.
8. Hadley, P.: Science News-letter, June 14, 1930.
   Hadley, P., Delves, E., and Klimek, J.: Filtrable Forms of Bacteria; Filtrable Stage in the Life History of Shiga Dysentery Bacillus, J. Infect. Dis. **48**: 1-159, 1931.
9. Schieblich, M.: Die Metschnikoffsche Theorie und der Einfluss der Kost auf Darmflora, Wachstum, Fortpflanzung, Gebaren und Blutbild der weissen Ratte; das Blutbild bei verschiedenen Kostformen und den dabei resultierenden Darmfloratypen, zusammenfassung, Zentralbl. f. Bakteriol **113**: 55-63, 1929.
10. Die Bedeutung der normalen Magendarmflora fur den tierischen Organismus, Berl. tierärztl. Wchnschr. **45**: 729-733, 1929.
11. Kulp, W. L.: Studies on Viability of L. acidophilus in Acidophilus Milk, Am. J. Pub. Health **21**: 873-883, 1931.
12. Rettger, L. F., and Cheplin, H. A.: A Treatise on the Transformation of Intestinal Flora, Yale Press, 1921.
13. Clendening, L.: Modern Methods of Treatments, St. Louis, 1931, The C. V. Mosby Co.
14. Cruickshank, R.: The Experimental Transformation of the Intestinal Flora, Brit. J. Exper. Path. **9**: 318-326, 1928.
15. Cruickshank, J., and Berry, D. W.: A Study of Bacillus acidophilus in Human Faeces, Brit. M. J. **41**: 944-553, 1924.
16. Hitzrot, L. H., and Lynch, F. B., Jr.: Determination of Effectiveness of Acidophilus Culture in Functional Disturbances of Colon, J. Lab. & Clin. Med. **15**: 548-553, 1930.
17. James, L. H.: Commercial Bacillus Acidophilus and Bacillus Bulgaricus Cultures and Preparations, J. A. M. A. **89**: 89-92, 1927.
18. Hughens, H. V.: Colonic Irrigation and Bacillus acidophilus Milk in Diarrhea, U. S. Naval Med. Bull. **22**: 691-692, 1925. A Biophysiotherapeutic Procedure in the Treatment of Nonmalignant Diseases of the Colon, U. S. Naval Med. Bull. **22**: 511-526, 1925.
    Norman, N. P.: Corrective Treatment of the Commoner Forms of Enterocolitis, Internat. Clin. **1**: 33rd series, 50-70, 1923.
19. Smith, R. P.: The Implantation or Enrichment of Bacillus acidophilus and other Organisms in the Intestine, Brit. M. J. **41**: 948-950, 1924.
20. Bergey, D. H.: Manual of Determinative Bacteriology, ed. 3, 1930, Baltimore, Williams & Wilkins Co.
21. Stitt, E. R.: Practical Bacteriology, Bloodwork, and Parasitology, ed. 8, 1927, Philadelphia, P. Blakiston's Son & Co.
22. Hiss, P. H., and Zinsser, H.: Textbook of Bacteriology, ed. 4, 1922, New York, D. Appleton & Co.
23. Ford, W. W.: Bacteriology, 1927, Philadelphia, W. B. Saunders Co.

24. Chambers, R.: New Micromanipulator and Methods for the Isolation of a Single Bacterium and the Manipulation of Living Cells, J. Infect. Dis. **31**: 334-343, 1922.
Kahn, M. C.: Chambers' Micromanipulator for the Isolation of a Single Bacterium, J. Infect. Dis. **31**: 344-348, 1922.
25. Kopeloff, N., and Beerman, P.: Modified Gram Stains, J. Infect. Dis. **31**: 480-482, 1922.
26. Mosse, K., and Happe, H.: Uber den Einfluss durch Milchsaure gesauerter Milch auf das Bakterienwachstum, Monatschr. f. Kinderh. **36**: 1-11, 1927.
27. Bass, C. C.: Transformation of the Intestinal Flora, Ann. Clin. Med. **1**: 25-32 1922.
28. Roos, C.: Cultural Characteristics of Lactobacillus Acidophilus, J. Lab. & Clin. Med. **12**: 1053-1058, 1927.
30. Reichart, E. L., and Davis, H. P.: Acidophilus Milk, Neb. Agr. Exper. Bull. **228**: 1-19, 1928.
31. Hauduroy, P.: Techniques de Culture des Formes Filtrantes Invisibles des Microbes Visibles, Compt. rend. Soc. de biol. **97**: 1392-1394, 1927.

A STUDY OF THE LACTOBACILLI MEDIA EMPLOYED*

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IN THE first paper of this series, it was shown that the members of the lactobacilli group may be arranged in a cycle according to the nature of the food supply and the conditions of growth (aerobic, anaerobic, facultative anaerobic, P_H value, and incubation temperature) which are characteristic for each bacillus. It was further shown that any member of the lactobacilli group may be changed into, or caused to assume, the morphology and cultural characteristics of any other bacillus in this cycle through a successive culturing on selective media. The object of this paper is to describe these various media.

The media employed to obtain the characteristic morphology of the lactobacilli located on the nonintestinal side of the cycle, were found described in literature. Whey and whey agar, prepared according to Rettger and Horton,[1] to which the variously indicated sugars were added, was employed. Kulp's tomato medium[2] was also used.

In determining the medium to be employed for the growth of the organisms listed on the intestinal side of the cycle, a careful study of the habitat of the individually isolated and recorded members of the lactobacilli group was made. The source of most of these bacilli was found to be the intestinal canal. Chemical analyses of the intestinal contents during digestion were studied and the following media developed:

MEDIA	ALPHA WHEY BROTH	DELTA WHEY BROTH	THETA WHEY BROTH
	(PER CENT)	(PER CENT)	(PER CENT)
Whey	94.1	94.1	94.1
Sugar	0.4 (lactose)	0.4 (maltose)	0.4 (glucose)
Casein	1.4	1.4	1.4
Albumin	1.95	1.95	1.95
Pepsin	1.95	1.95	1.95
Sodium citrate	0.2	0.2	0.2

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Tomato nutrient broth was prepared by dissolving 8 grams of dehydrated nutrient broth (Difco) and 8 grams of sodium chloride in 500 c.c. of water. Then 200 c.c. of tomato juice were added and the whole was made up to 1000 c.c. It was then filtered through cheesecloth, adjusted to a P_H of 5.2, and sterilized in the autoclave.

LACTOBACILLUS ACIDOPHILUS MEDIA

The natural habitat of the Bacillus acidophilus is the intestinal tract of an infant during the early period of lactation. Many investigators have reported the intestinal tract of a newborn babe free from bacteria, but shortly after birth a bacterial flora develops which contains organisms of the lactic acid group. After the infant is weaned the intestinal flora changes, and putrefactive types of bacteria appear. Thus, the most natural food supply for the bacillus acidophilus would be digested human milk at a P_H of the intestine of an infant during the early stages of lactation. This would require a medium similar in composition to human colostrum.

A chemical study of the early milk of lactation (colostrum) showed some surprising differences in composition when compared to normal human milk. The outstanding differences were the higher value for total solids and total proteins and the relatively lower lactose value. An analysis of colostrum compared to normal human milk is given in Table I. Another important factor in the composition of human colostrum is the relatively large amount of zinc ion. Zinc salts have been reported to coagulate milk more readily than any other kind of inorganic salt (Andreitchewa[3]). They have a peroxydase function but cannot function as an oxydase or catalase. They exhibit an unfavorable effect upon the action of protolytic ferments (pepsin and trypsin). Zinc opposes the action of catalase as shown by Zlataroff.[4] Contrary to general belief, zinc is not toxic in concentrations up to 0.5 parts per 100 (Heller and Burke)[5] and previously reported poisoning were due to impurities (Batchelor, Fehnel, Drinker, and Thompson[6]).

TABLE I

ANALYSIS OF MILK

KIND	HUMAN			COW	
	NORMAL	NORMAL AVE.	COLOSTRUM	NORMAL	COLOSTRUM
SOURCE	1	2	3	4	5
	(PER CENT)	(PER CENT)	(PER CENT)	(PER CENT)	(PER CENT)
Specific gravity	1.0298	1.0313	1.0330	1.0313	1.042
Water	87.58	88.20	82.05	87.27	75.07
Fat	3.74	3.33	4.40	3.68	3.97
Lactose	6.37	6.80	2.64	4.94	2.28
Total Proteins	2.01	1.50	9.96	3.39	17.18
Casein	0.80	1.00	3.11	2.88	4.19
Albumin	1.21		6.85	0.51	12.99
Total Ash	0.30	0.20	0.95	0.72	1.53
Total Solids	12.42	11.80	17.95	12.73	24.93

(1) Heineman, Milk. (2) Average of 10 analyses made by one of the authors. (3) Single analysis made by one of the authors. Calculations based on 2 and 5 gave a result in very close agreement with 3. (4) Heineman, Milk. (5) Heineman, Milk.

From a consideration of the data we developed three media for the culturing of the lactobacilli. It was found necessary to add 0.02 per cent of zinc in the form of the chloride (0.04 per cent) to the digested artificial media when culturing a lactobacillus which would give the morphological and cultural characteristics of B. acidophilus. Whether the zinc aided in the coagulation and digestion by stimulating the action of the peroxydases or by limiting the production of the amino acids through its inhibiting action on the protolytic ferments, pepsin and trypsin, is not known. Zinc will accelerate the coagulation of a partially decalcified milk by rennin.

The medium is adjusted to P_H 7 and autoclaved.

S AND W DIGESTED MEDIUM

S and W medium was digested with hydrochloric acid (P_H 2) for 4 hours. Adjusted to P_H 8 with NaOH and then digested for 4 hours with pancreatin. Adjusted to P_H 7 and autoclaved.

S AND W MEDIA

	HUMAN COLOSTRUM	FAT AND H_2O FREE BASIS	WET BASIS	500 C.C. OF MEDIA
	(PER CENT)	(PER CENT)	(PER CENT)	GRAMS
Lactose	5.44	45.15	8.11	40.6
Casein	1.45	12.03	2.16	10.8
Albumin	3.27	27.13	4.87	24.4
Peptone	1.50	12.45	2.24	11.2
Fat	5.90			
Citric Acid		0.82	0.15	0.5
Lactic Acid		0.95	0.17	0.9
Zinc (as $ZnCl_2$)				0.04
Zn.		0.01	0.002	
NaH_2PO_4		0.80	0.15	0.8
$CaCl_2$		0.65	0.12	0.6
Ash	0.39			
Total Solids	17.95	99.99	17.972	
Water	82.05		82.028	500 c.c.

S AND W SUCROSE MEDIUM

Sucrose was substituted for lactose in S and W medium and the digestion carried out as indicated under S and W digested medium.

When correctly prepared, the S and W medium after sterilization, had the consisteney and color of chyme and was odorless. Amino acid splitting organisms produced a black color and putrefactive odor in twenty-four to forty-eight hours at an incubation temperature of 37° C.

TOMATO DIGEST MEDIUM

Lactose or glucose	13.51 per cent
Casein	3.53
Albumin	8.11
Peptone	3.73
Water	71.12

The above mixture is hydrolyzed with 0.4 per cent hydrochloric acid for 4 hours at 37° C., and adjusted to P_H 7. It was then digested with pancreatin

for 4 hours at 37° C. To 60 parts of the above digested medium 40 parts of the following solution were added and the whole adjusted to P_H 7 and autoclaved.

NaCl	0.9 grams
NaH_2PO_4	0.15
$CaCl_2$	0.12
$ZnCl_2$	0.01
Tomato Juice (filtered)	38.8

REFERENCES

1. Rettger, L. F., and Horton, G. D.: Treatise on the Transformation of Intestinal Flora, Yale Press, 1921.
2. Kulp, W. L.: An Agar Medium for Plating Lactobacillus Acidophilus and Lactobacillus Bulgaricus, Science **66**: 512-513, 1931.
3. Andreitchewa, M.: Contribution a la Chimie Biologique du Zinc, Bull. Soc. chim. biol. **12**: 45-57, 1930.
4. Zlataroff, As.: Le Zinc et le Cancer, Bull. Soc. chim. biol. **12**: 44, 1930.
5. Heller, V. G., and Burke, A. D.: Toxicity of Zinc, J. Biol. Chem. **74**: 85-93, 1927.
6. Batchelor, R. P., Fehnel, J. W., Thompson, R. M., and Drinker, K.: A Clinical and Laboratory Investigation of the Effects of Metallic Zinc, of Zinc Oxide, and of Zinc Sulphide Upon the Health of Workmen, J. Indust. Hyg. **8**: 322-363, 1926.
7. Stitt, E. R.: Practical Bacteriology, Bloodwork and Parasitology, ed. 8.
8. Rotch, T. M., and Kendall, A. I.: A Preparatory Study of the Bacillus Acidophilus in Regard to Its Possible Therapeutic Use, Am. J. Dis. Child. **2**: 30-38, 1911.

THE BLOOD PRESSURE LOWERING EFFECT OF BISMUTH SUBNITRATE BY MOUTH IN NORMAL AND INCREASED ARTERIAL TENSION*

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THE nitrite effects of enteric bismuth subnitrate were first manifested clinically in toxic symptoms which followed its early application in x-ray visualization of the gastrointestinal tract. Sailer and Pancoast[1] presented several occurrences of such intoxication after oral administration. Rautenberg[2] described the symptomatology after enema in an almost fatal case. Prior[3] reported it in an infant after over-dosage. Further instances were adduced by Meyer,[9] Zabel,[4] Phillips,[5] and Stieglitz.[15]

Chemicopathologic findings in fatalities demonstrated the intermediation of nitrite and located the site of its production. Hildebrand[6] referred to two infant deaths after anal injection. In Benneke and Hoffmann's[7] premature infant, necropsy showed bismuth in the colon and rectum, a brownish discoloration of the tissues, and methemoglobinemia. Böhme's[8] infant revealed the presence of nitrite in the blood and pericardial fluid, and an absence of bismuth from the blood and liver. In Meyer's[9] adult with intestinal tuberculosis nitrite was present in the feces between two strictures of the small intestine. Nowak and Gütig[10] reported the death of an adult after enema. In Zadek's[11] case of gastric carcinoma with achlorhydria, bismuth was distributed throughout the intestinal canal, nitrite was present in the content of all segments, but especially of the lower portions, of the small intestine, and nitrate was demonstrable in the large intestine. Thus bismuth was present in the colon;[2, 5-11] nitrite was produced in the large intestine, and under abnormal conditions in the small intestine,[9, 11] and circulated in the blood;[8, 9] and methemoglobin developed in the blood[2, 6] and tissues.[7-10]

The capacity of feces to produce nitrite from bismuth subnitrate was substantiated experimentally. Böhme[8] found that aqueous suspensions of the feces of children, and, less consistently and in a lesser degree, of adults, after admixture of bismuth subnitrate, on incubation gave strongly positive nitrite reactions, and on injection into the alimentary canals of cats and rabbits resulted in the excretion of nitrite in the urine. Zadek[11] incubated the feces in diverse dietary modifications and pathologic conditions with one-tenth its weight of bismuth subnitrate, and detected nitrite in distilled water extracts of a fair proportion of adult specimens and in the greater number of specimens of children. The administration of bismuth subnitrate to a child with a gastrointestinal disorder on one occasion resulted in nitrite-containing feces. With this exception the administration of large daily doses of bismuth subnitrate resulted in feces, which was nitrite negative on passage, and which remained nitrite negative after

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incubation. The capacity of such feces to produce nitrite on addition of bismuth subnitrate and incubation was unimpaired.

Nitrite poisoning by enteric bismuth subnitrate was duplicated in animal experiments by Nowak and Gütig[10] who elicited the complete syndrome in cats.

The material was reviewed by Beck,[12] Zollinger,[13] and Mayer and Baehr,[14] who accepted acute nitrite poisoning following the internal administration of bismuth subnitrate as proven.

BISMUTH SUBNITRATE IN ARTERIAL HYPERTENSION

The nitrite effect of bismuth subnitrate by mouth, as exerted by small doses, has been applied therapeutically in arterial hypertension by Stieglitz.[15-18] In essential hypertension, more especially when uncomplicated by advanced arteriolosclerosis, the average systolic pressure in large series reverted far toward the maximum normal for the average age, and the diastolic approached normal. In the hypertension of pregnancy the blood pressure elevation was moderated and its progress toward parturition arrested in the large majority of cases. While the data were not controlled with regard to an adequate regulated period of pre-treatment observation, the effect of nonspecific medication, uniform frequency of visit during the treatment and pre-treatment periods, and a standardized procedure in determining the blood pressure, in essential hypertension,[19, 20] or by parallel series without specific medication in the hypertension of pregnancy, these results are suggestive.*

The blood pressure reduction following the administration of bismuth subnitrate by mouth might be anticipated to be within certain limits dependent on the dosage. The procedure was accordingly adopted of increasing the dosage by steps until the maximal effect was attained. Such administration of graded doses gave indications of a clearcut relationship between dosage and decrease in blood pressure.

CASE REPORT

History.—H. C. C., male, aged fifty-three, an engineer engaged in the transaction of clerical business, who had previously been free from major illnesses, and was in apparently good health, in the early afternoon of June 24, 1930, suddenly felt himself staggering. During the course of the following morning an abrupt violent attack of nausea and vomiting occurred. The nausea lasted throughout the day. Dizziness on walking persisted. Three days from the onset of symptoms the blood pressure was 200 systolic, 110 diastolic (Chart 1). The maximum concession made in restricting activity consisted in coming to the office in mid-morning, and leaving by noon or mid-afternoon. The patient was regular in habits, ate sparingly, little meat, largely vegetables and starches, drank a quart of buttermilk a day, smoked heavily, and habitually took no exercise. A solution of potassium iodide, gtt. viii, later gtt. x, t. i. d., was prescribed, and subsequently supplanted by ferric iodobehenate, gr. viii, t. i. d., with such effect that the coryza of iodism by several weeks outlasted their administration. Phenobarbital, gr. ss., t. i. d., was taken over a long period without subjective notice. Meanwhile the blood pressure mounted to 224 and subsided to 204 systolic, and mounted to 124 and found a level at 102 diastolic. The dizziness had in a little over a month receded to infrequent slight spells, which decreased until they remained only in the traces of

*As the result of the study of a small series in which these factors were taken into account Ayman[21] more recently concluded that bismuth subnitrate in such dosage exerts no demonstrable effect in essential hypertension, although in the majority of cases the frequency of observation both in the entire period of previous observation and in the immediate fore-period was greater than in the treated period, which might be prejudicial to the appearance of a bismuth subnitrate effect.

some passing uncertainty on rising, which finally disappeared. Thenceforward the course was asymptomatic. Normal activity was resumed, except that an occasional freedom was accepted in coming to work late and leaving early, and extremes of over-exertion were avoided. Cucurbocitrin[21-27] 50 mg., t. i. d., was taken without altering the trend of the systolic pressure, but when the blood pressure readings were in addition made at weekly instead of biweekly intervals, it fell 10 points,[19, 20] only to rise 5 points when the blood pressure was not taken for a month; the diastolic pressure fluctuated about a mean of 104 during this period. All medication was discontinued. The patient, by direct inquiry and by various shifts, had secured the opinions of different physicians as to the eventualities and hazards of his malady, and retailed their diverse pronouncements with uniform humor and delight, seemed happy to be in a position to observe the course of his disease and content to accede to whatever might be its outcome, and felt considerable amateur interest in the various proposed methods of therapy, encouraged their trial, and was ready to cooperate faithfully in their prosecution.

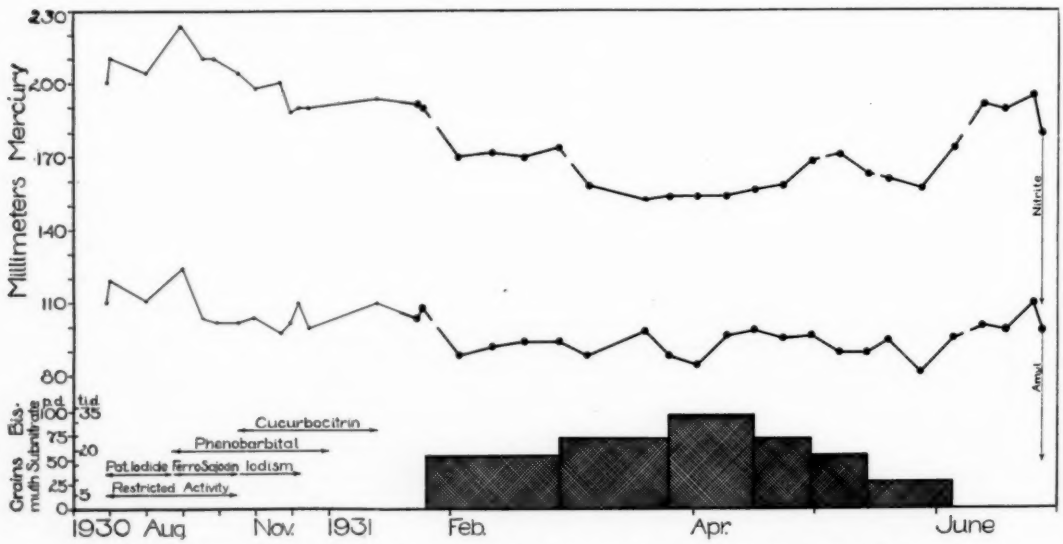

Chart 1.—Graphic case report: arterial hypertension treated with bismuth subnitrate.

Physical Examination.—Middle aged man, average height and weight, approximating athletic habitus, fair sedentary musculature, some want of turgor in the skin and subcutaneous tissues of the face, slight pallor of the mucous membranes. Palpable arteries, compressible, nonresistant, not tortuous. Retinal arterioles, pale, moderately reduced caliber, slightly roughened outline, straight, branching at sharp angles, stiff and rigid looking; veins indented by the passage of the arterioles over them and bulging slightly.[28, 29] Apex beat palpable to the midclavicular line in the sixth interspace. Inconstantly a soft, but somewhat roughened, blowing nontransmitted systolic murmur at the apex.

Laboratory Tests.—Urine; routine specimens, specific gravity, 1015-1034; consistently a trace albumin; infrequent hyaline casts. Blood chemistry: urea nitrogen 10.6, uric acid 3.5, glucose 92 mg. per 100 c.c. Blood Wassermann: negative. Basal metabolic rate: plus 4 per cent by the Boothby and Sandiford revision of the Du Bois standards.

Therapeutics.—The blood pressure was determined after rest periods of some minutes to a half hour the duration of which depended upon office exigencies. Successive readings were made, usually to the number of ten, during an interval of several minutes, or until they no longer tended downward but fluctuated on a level. The duplicate readings at this level were averaged. This conduced to securing comparable, partially controlled, semibasal pressures.

The blood pressure was determined weekly. Most readings were made on Wednesdays, but a few on Mondays and Fridays.[30] All were taken in the mid- or late afternoon.[31]

The average range between the maximum and minimum readings at a sitting was 26 systolic, 8 diastolic.[31-33] On one occasion after the patient had for the previous half hour been unable to escape the annoyance of an insistent salesman the range was 46 systolic, though but average range diastolic.

The initial blood pressure determinations located a mean at 191 systolic, 106 diastolic.

No change in the dietic, hygienic, or psychic regimen was advised. The continuance unmodified of the prevailing routine was encouraged.

Bismuth subnitrate, in capsules or chartulae, t. i. d., apportioned approximately q. 8 h. to conduce to an even distribution through the twenty-four hours, was prescribed in graded dosages over a period of ten months.

The initial dosage of gr. xviii was continued for over one month. During this interval the systolic pressure assumed a mean level of 172. The dosage was increased to gr. xxiv for one month, further increased to gr. xxxii for three weeks, and returned to gr. xxiv for two weeks. During these intervals the systolic maintained a mean of 157. The dosage was returned to gr. xviii for two weeks. During this interval the pressure reverted toward its earlier level under this dosage with readings of 171 and 163. The dosage was reduced to gr. ix for three weeks. On the first and second visits during this interval the rest period was prolonged, and relaxation was very complete, but on the third it was of usual length. The respective readings were 160, 157, and 173. Some partial remission in the hypertension[33, 34] may have entered into this irregularity.

The diastolic pressure assumed a mean level of 92 about which it fluctuated as long as bismuth subnitrate was continued.

During the treated period the average lability range was 14 systolic, 7 diastolic. After several hours of rush and strain, unmoderated by any intervening rest period, the range was on one occasion 36 systolic, 19 diastolic, even though the prevailing systolic level was not reached, but on another occasion, when neither the prevailing systolic nor diastolic was reached, no more than average. Once after a few moments of heedlessly vigorous exercise a fall of 65 systolic, 24 diastolic, occurred in regaining the prior level over an interval of seven minutes.

Bismuth subnitrate was discontinued for three weeks. The blood pressure reverted to approximately its initial mean level with 188 systolic, 103 diastolic.

During the combined untreated periods, the mean blood pressure was 189 systolic, 104 diastolic. During the interval of maximal effective dosage of bismuth subnitrate the mean systolic was 157—a reduction of 32, or 16.9 per cent. On decreasing the dosage the systolic pressure tended to revert to the level it has previously assumed with such dosage. Throughout the period of bismuth subnitrate administration the mean diastolic was 92—a reduction of 12, or 11.5 per cent. On discontinuing bismuth subnitrate the blood pressure resumed its initial level.

When the blood pressure is plotted against the dosage of bismuth subnitrate and smoothed median curves drawn (Chart 2), the systolic pressure and its percentile reduction follows a skew sigmoid curve which runs into a horizontal line beyond gr. xxv, the diastolic a hyperbolic curve which runs into a horizontal line beyond gr. x.

As a comparative test of the maximum blood pressure reduction resulting from acute extreme vasodilatation, the patient vigorously and exhaustively in-

haled 5 minims of amyl nitrite, with the result that the blood pressure at the approximate instant of maximal blushing and pulse acceleration[35] read 108 systolic, 43 diastolic. The maximal reduction obtainable with bismuth subnitrate was thus less than the maximum reduction effected by amyl nitrite. The limitation in the maximal effects of bismuth subnitrate was, therefore, not due to a nondilatability of the hypertensive or sclerosed arterioles.

Freshly voided urine invariably tested negative for nitrite.

With the larger dosages some slight temporary intestinal retardation was experienced, which was readily counteracted by occasional small doses of magnesium oxide, and later in some way compensated without it.

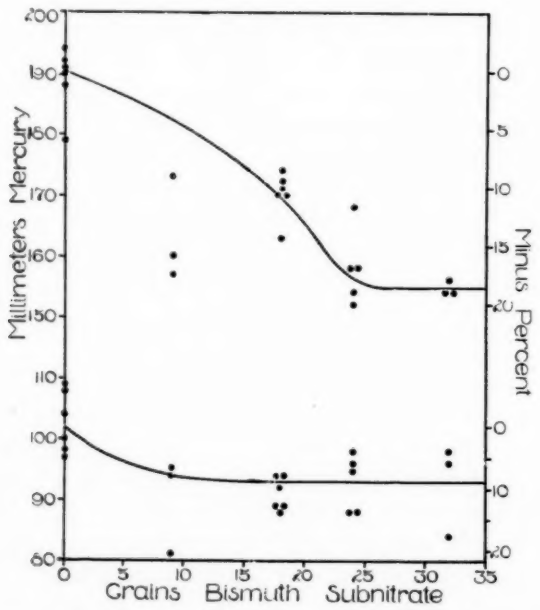

Chart 2.—Relationship of dosage of bismuth subnitrate to blood pressure in case of arterial hypertension.

Even when the fourth pound of bismuth subnitrate was being consumed, the gums gave no evidence of a metallic line, although there was an early pyorrhea of the lower incisors to furnish the requisite sulphide.

The administration of the maximal effective dosage of bismuth subnitrate was resumed: the blood pressure resumed the level it had previously maintained under this medication.

Diagnosis.—Essential hypertension: moderate blood pressure elevation, hypertensive blood pressure lability,[31-34] retinal arterioles of hypertension with arteriosclerosis,[28, 29] moderate left ventricular hypertrophy, slight renal involvement, asymptomatic.

Summary.—Bismuth subnitrate by mouth was accompanied by a reduction of the systolic pressure which increased with dosage until the maximal was reached, a uniform reduction in the diastolic pressure, and a decrease in blood pressure lability.

Bismuth subnitrate by mouth thus appears to reduce the blood pressure in arterial hypertension and inhibit its lability. The greatest effect obtainable is

limited. This limitation is not attributable to an inability of the arterioles to dilate. The blood pressure reduction might be conditioned by the intermediary processes through which bismuth subnitrate exerts its nitrite effect. The limitation might be secondary either to a limitation in the capacity for the conversion of bismuth subnitrate into nitrite in the alimentary canal, or to a limitation in the response of the hypertensive arterioles to nitrite produced in such quantity and at such a rate.

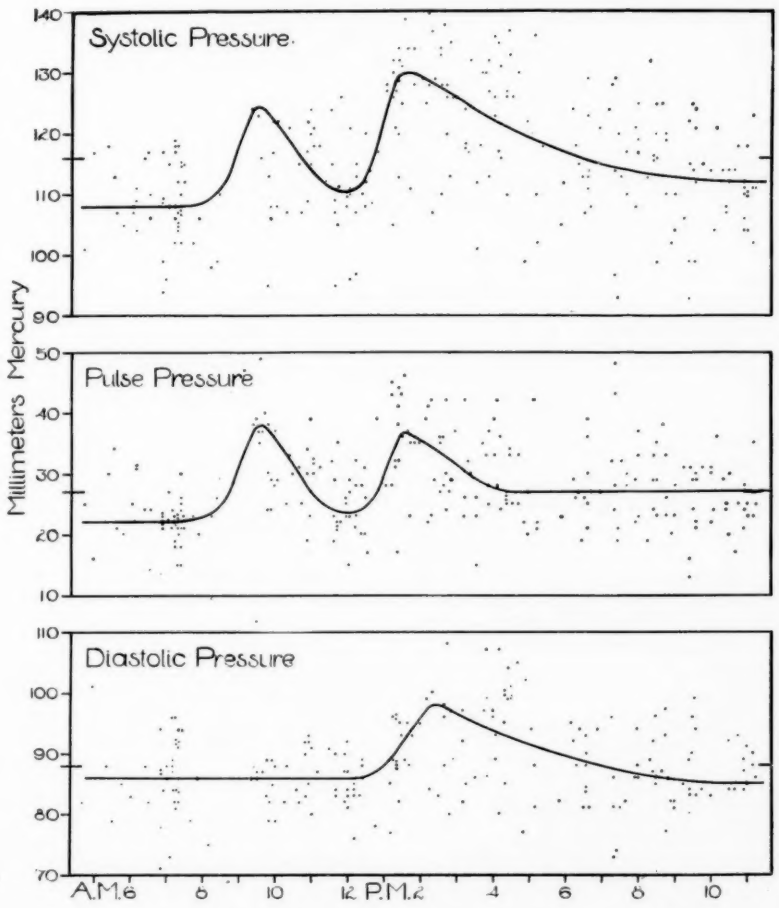

Chart 3.—Diurnal blood pressure variation in normal arterial tension.

Investigation of the temporal course and relative extent of the blood pressure lowering effect of graded dosages of bismuth subnitrate should demonstrate the potentialities and define the limitations inherent in this means of medication.

EXPERIMENTAL

Since the nitrite effect of bismuth subnitrate by mouth might not supervene for some hours and should continue over an indefinite period, and inasmuch as the blood pressure traverses a diurnal cycle,[36, 37] it is not feasible to determine

TABLE I

Bismuth Subnitrate by Mouth in Normal Arterial Tension

CONTROL	BISMUTH SUBNITRATE				BISMUTH FECES		URINARY NITRITE	SUBMEDIAN BLOOD PRESSURE									
									SYSTOLIC					DIASTOLIC			
DATE	DOSAGE	DATE	HOUR		INITIAL APPEARANCE	COMPLETE EVACUATION		OBSERVATION PERIOD	PERIOD	MEDIAN		MAXIMUM		PERIOD	MEDIAN		MAXIMUM	
			A. M.	P. M.	HR.	HR.		HR.	HR.	MM. HG.	PER CENT.	HR.	MM. HG.	PER CENT.	HR.	MM. HG.	PER CENT.	HR.	MM. HG.	PER CENT.
1931 Apr. 22	GR. 150	1931 May 26	6:30		28[2]	77[2]		0 –16	5/6 –12[3]	11	9.6	4 11/12	23	20.7	1[1] – 8 11/12	3	3.5	4 11/12	14	16.3
23		June 7	7:10		26[4]	77[2]	negative	0 –15 1/12[5]	0 –14[3]	17	14.9	7 5/12	35	27.6	0 –15 1/12	9	10.5	7 5/12	26	26.5
24	100	May 14	1:40		31[1]	55		5[3] –21 7/12	5[3] –16[1]	12	10.5	8 5/6	21	17.8	5[3] –16	4	4.7	8 5/6	8	9.3
25		June 11	5:50		27[1]	58		0 –16[3]	[3] –12[3]	14	12.3	7[3]	29	22.3	0 –11 1/12	8	9.4	8 7/12	14	14.3
26[1]	75	May 7	1:40		13[1]	62[1]		5[3] –21	5[3] –14[1]	10	8.8	8	27	21.8	5[3] –10[1]	4	4.7	8	9	10.5
29		30	6:10		28[3]	48[1]	negative	0 –15 5/6[5]	5/6 –11	7	6.1	3 1/12	23	18.7	0 –12 1/12	10[1]	12.3	8 11/12	16	16.7
May 1	50	Apr 27	12:00		21[1] (16[1])[4]	69[1]		0 –10[3] 19 –24	[3] – 3[3]	11	9.6	3	18	14.3	[3] – 3 7/12	10	11.7	3	12	12.4
3		29		10:00	35[1]	59		9[1] –23 11/12	9[1] –18 11/12	2[1]	2.2	12[1]	16	13.4	9[1] –12[3]	2[1]	2.9	12[1]	5	5.8
5		June 5	6:55		28[1]	50[1]		0 –16 1/12	[1] –16 1/12	7	6.1	3[1]	22	18.3	[1] –16 1/12	7	8.2	3[1]	11	12.8
6								24 5/12 –38 5/6[5, 6]	24 5/12 –36 11/12	12	10.5	27[1]	24	20.2	24 5/12–38 5/6	11	12.9	33[1]	17	18.3
8	25	May 1		10:30	41[1]	58[1]		8 –24[6]		1	.9					–2	–2.4			
11		4	5:30		34[1]	54[1]		1[3] –17 1/12	1[3] –10	6	5.3	4[1]	9	7.5	1[3] – 7 5/12	2[1]	2.9	4[3]	4	4.6
13		24	6:45		26[1]	50[1][2]	negative	0 –15[3][5]	1[1] –13[3]	10	8.8	3 5/6	15	12.8	3 –14[1]	5	5.9	8	18	18.4
15	20	May 16	6:40		34	34		0 –15 11/12[5]	11/12–12[1]	6	5.3	3[1]	22	18.0	1[1] –13[1]	6	7.1	3[1]	14	16.3
17		June 1	3:55		31[1] (16[3])[4]	53[1]		1/12 –18[1]	1[1] – 6[1]	6[1]	5.7	2[3]	11	10.2	1/12–10[1]	6	7.1	7 5/6	10	11.6
19		17	7:20		26[1]			0 –15[1]	0 – 4 5/12	6	5.3	3[1]	21	17.9	0 – 4[1]	6	7.1	3[1]	13	15.1
21				2:20		44[1]			7 11/12–10 7/12	11	9.6	8 11/12	17	14.0	6 –11	5[1]	6.5	8[1]	9	9.5
23	15	May 9	7:45		25	35		0 –14 11/12[5]	0 –11 1/12	6[1]	5.7	7 1/12	34	27.0	5[3] –12[1]	3	3.5	7 1/12	15	15.5
25								23[1] –38[3][4, 6]	23 11/12–38[3]	11	9.6	30	22	16.9	23[1] –38[3]	6	7.1	34 1/12	15	16.3
27		22	5:35		27[3][2]	57[1]	negative	0 –17[1]	1 – 9	6	5.3	3 11/12	18	14.5	2 7/12– 9[1]	4[1]	5.3	6[1]	8	9.3
29	10	May 17		10:00	33	33		7 11/12–23 5/6		–1	–.9					–3	–3.5			
31		28	7:25		28[3]	74[1][3]		0 –14 11/12	7/12 – 5[1]	7[1]	6.6	3 11/12	9	8.0	1[1] – 5[1]	4[1]	5.3	3 11/12	6	7.0
June 2		June 9	6:55		29[1] (19[1][4])			0 –14[1]	7/12 – 9 1/12	9	7.9	4 1/12	17	15.0	1[1] –10[1]	7	8.2	4 1/12	11	12.8
4	5	May 11		11:00	13[1] (15[1])[4]	59[1]		7[1] –24		–[1]	–.4					–2[1]	–2.9			
8		20	4:40		26[1]	36[5]/6	negative	0 –18 1/12	7/12 – 6 7/12	4	3.5	4 11/12	18	14.5	1 1/12– 5 7/12	6	7.1	4 11/12	9	10.5
10		June 3	7:30		26	50]		0 –15 1/12	2 1/12 – 5 5/12	4	3.5	2 7/12	13	10.7	[3] – 8	4	4.7	4 11/12	7	8.1

[1]Daylight Saving Time. [2]Negative for Nitrite. [3]Estimated: no defined demarcation. [4]Initial appearance of recognizable vegetable remnants. [5]Saturday or Sunday. [6]Putrefactive Feces.

the pressure, administer the drug, subtract subsequent determinations from the original reading, and attribute the difference to the drug, assuming that without it the blood pressure would on the average have remained constant, as with the sooner and more briefly acting members of the nitrite group.[38-40] Since the

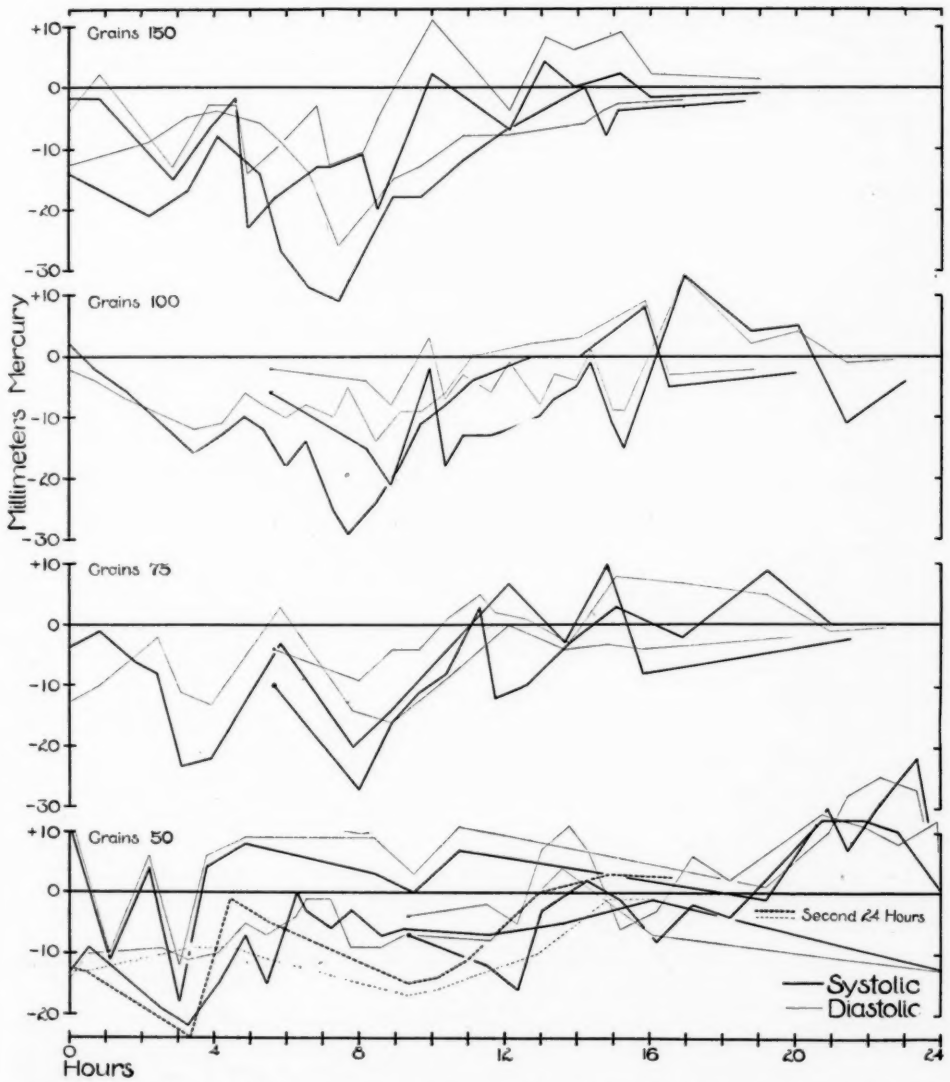

Chart 4.—Deviation of blood pressure from median after bismuth subnitrate by mouth in normal arterial tension.

blood pressure is less variable and erratic than in hypertension[37] more consistent data should be obtainable in normal arterial tension. Since it would be necessary to follow the blood pressure closely over a long period in order to establish adequate controls and conduct sufficient tests, it seemed more practicable to pursue the experiment in person.

On five consecutive and twenty-one alternate days (Table I) the blood pressure was determined so far as practicable once during every waking hour. The pressures were taken with only such interruption of activity as was necessary to their taking. Each pressure was the average of five successive readings. While each reading was being noted down, the air was

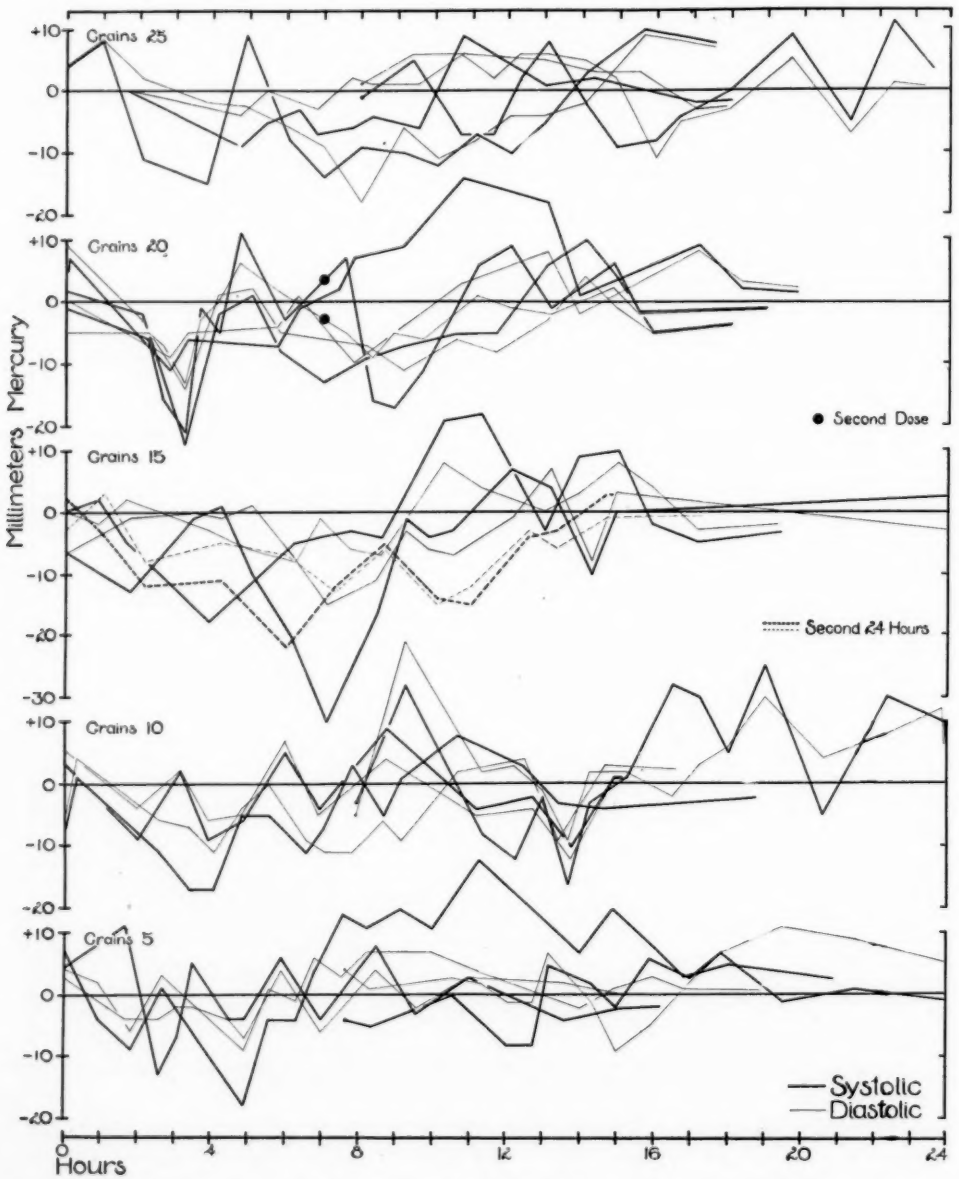

Chart 5.—Deviation of blood pressure from median after bismuth subnitrate by mouth in normal arterial tension.

allowed to escape freely from the cuff, so distal circulation might be reestablished. When indicated the procedure was modified to insure securing a representative value for the prevailing pressure. Occasionally a low initial reading resulted from the tendency to hold the breath and prolong inspiration[41] while watching the pointer or the mercury column and waiting to

hear the changes in the sounds. Infrequently some external disturbance or interruption caused a few high readings. Such aberrant readings were cancelled. Whenever the successive readings appeared to be unusually divergent additional readings were made. The several readings were averaged to the nearer millimeter; the time was recorded to the nearer twelfth-hour. The diastolic, pulse, and systolic pressures were plotted against the hour of the day on scatter diagrams, and smoothed curves drawn through the median pressures of consecutive hours (Chart 3). The establishment of the trend and variation of the diastolic and systolic pressures through the day constituted a basis for comparison of the pressures following the administration of bismuth subnitrate.

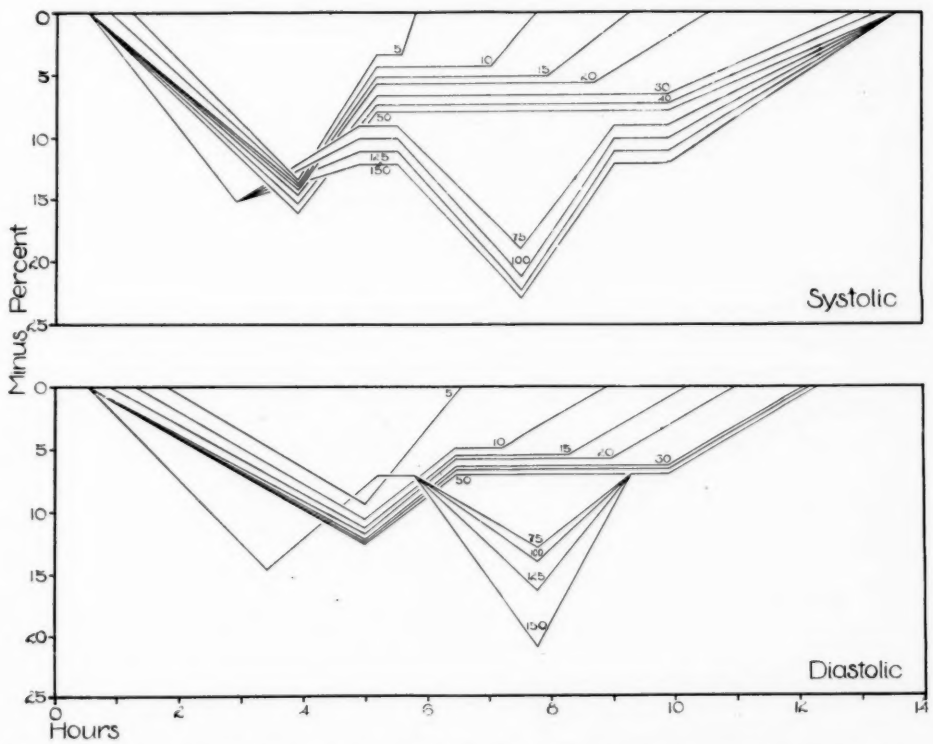

Chart 6.—Blood pressure lowering effect of bismuth subnitrate by mouth in normal arterial tension.

Graded doses of bismuth subnitrate, the supernatant fluid of distilled water suspensions of which tested negative for nitrite, were taken as powders, followed by a little water, and, in the bulkier amounts, by a few mouthfuls of food, to aid in swallowing, in random sequence, at the beginning of the intervening days or during the anteeedent nights (Table I). The blood pressure was determined at frequent intervals. Each diastolic and systolic pressure was converted into millimeters plus or minus deviation from the median pressure for the same hour. The successive deviations with each dose were plotted against hours from administration and connected by straight lines (Charts 4, 5). From the original data and these charts, the period of submedial pressure, the median deviation in millimeters during this period and its percentile divergence from the median of the pressures of the trend on successive hours, the maximum deviation in millimeters and per cent, and the time of its occurrence, systolic and diastolic, in each test, were derived (Table I). These and additional statistics were generalized by plotting the times of beginning deviation, maximum deviation, discontinuance of median deviation, and cessation of deviation, and the maximum and median deviation in both milli-

meters and per cent,[39] systolic and diastolic, against dosage, and calculating averages or drawing smoothed curves as indicated. Readings from these averages and trends were recorded (Table II) and incorporated in a schematic diagram (Chart 6).

Throughout the period of investigation all stools were described with respect to time of passage, gross appearance, presence of recognizable vegetable remnants, and occurrence of bismuth discoloration. The number of hours after the administration of a dose of bismuth subnitrate of the initial appearance and of the complete evacuation of the corresponding segment of bismuth-greyed or -blackened feces was recorded (Table I). Tests for nitrite were in some instances made on filtrates of aqueous suspensions of bismuth-containing stools.

TABLE II

BLOOD PRESSURE LOWERING EFFECT OF BISMUTH SUBNITRATE BY MOUTH: NORMAL ARTERIAL TENSION

PRESSURE	DOSAGE	ONSET	PRIMARY MAXIMUM			SECOND MAXIMUM			LEVEL MAINTAINED			CESSATION
	gr.	hr.	hr.	mm. Hg	per cent	hr.	mm. Hg	per cent	mm. Hg	per cent	hr.	hr.
Systolic	5	1½	4	15.4	13.8				4.0	3.5	5½	5¾
	10	¾	4	15.7	14.0				5.1	4.5	7	7¾
	15	½	4	16.0	14.2				6.0	5.3	7¾	9¼
	20	½	4	16.4	14.3				6.7	5.9	8¾	10½
	25	½	4	16.8	14.4				7.3	6.4	9½	11¾
	30	½	4	17.1	14.7				7.8	6.9	9¾	12¾
	40	½	4	18.1	15.3				8.6	7.5	9¾	13¼
	50	½	4	19.4	16.1				9.2	8.1	9¾	13½
	75	½	3	19.0	15.2	7½	23.4	19.1	10.4	9.1	9¾	13½
	100	½	3	19.0	15.2	7½	26.0	21.3	11.6	10.2	9¾	13½
	125	½	3	19.0	15.2	7½	27.7	22.4	12.8	11.2	9¾	13½
	150	½	3	19.0	15.2	7½	28.8	23.0	14.0	12.3	9¾	13½
Diastolic	5	1¾	5	8.0	9.4							6½
	10	1¼	5	9.5	10.6				4.4	5.2	7	8¾
	15	1	5	10.2	11.4				4.8	5.7	8¼	10
	20	½	5	10.6	11.8				5.0	5.9	9	11
	25	½	5	10.9	12.1				5.3	6.2	9½	11½
	30	½	5	11.1	12.3				5.4	6.4	9¾	12
	40	½	5	11.2	12.5				5.7	6.7	9¾	12
	50	½	5	11.3	12.6				6.0	7.1	9¾	12¼
	75	½	3½	13.0	14.7	7¾	11.8	13.0	6.0	7.1	9¾	12¼
	100	½	3½	13.0	14.7	7¾	12.8	14.1	6.0	7.1	9¾	12¼
	125	½	3½	13.0	14.7	7¾	14.9	16.4	6.0	7.1	9¾	12¼
	150	½	3½	13.0	14.7	7¾	19.0	21.0	6.0	7.1	9¾	12¼

For alternate dosages of bismuth subnitrate urine specimens were collected from the preceding hour to the twenty-fourth hour after administration and saturated with an excess of chloroform to prevent bacterial decomposition. To test for nitrite 0.5 c.c. of sulphanilic acid and of a-naphthylamin acetate reagent[42] were added to 1.0 c.c. of each specimen. The reaction proceeded in the presence of chloroform. Under the conditions the test was sensitive to from 0.0001 to 0.0002 mg. $NaNO_2$ per c.c. The nitrite content of urine did not alter appreciably during such intervals as elapsed between passage and examination. The specimens were measured and their twenty-four hour volumes totaled.

Supplementary tests were undertaken to estimate the sodium nitrite equivalents of the blood pressure lowering effects of bismuth subnitrate by mouth, and to secure comparative information on nitrite excretion with the two drugs. During the morning hours when the blood pressure maintains a level (Chart 3), its height was determined, graded dosages of sodium nitrite, tablets of which were freshly dissolved in small amounts of water, followed by the

rinsings of the glass, taken, and the blood pressure read every few minutes until its fall had passed a maximum. The systolic and diastolic reductions expressed as percentages of the original pressures[39] were plotted against dosage and smoothed curves drawn. Urine specimens were collected for the first half hours following the administration of sodium nitrite and for several subsequent hours (and in the first instances through twenty-four hours). Their volumes were measured. To 1.0 c.c. of each specimen 0.5 c.c. of each reagent was added. The tint appearing in a positive test lighted through an equal depth of distilled water was matched with that of sodium nitrite standards viewed through a like volume of the urine specimen equally diluted. The nitrite content of the single specimens and of the several specimens following each dose were computed. The sodium nitrite excretion in mg. and per cent, and the actual and graduated systolic and diastolic blood pressure reductions were recorded (Table III).

DIURNAL BLOOD PRESSURE CYCLE

The blood pressure traverses a diurnal cycle. In a group of college students in whom the blood pressure was followed during an evening and a day, Weysse and Lutz[36] found a slight tendency toward a lowering of the diastolic pressure through the day, and a gradual increase in the systolic and pulse pressures, which, in addition, rose immediately after the ingestion of food and gradually declined till the beginning of the succeeding meal. In a group of ambulatory ward patients in whom the blood pressure was taken hourly during twenty-four hours, Mueller and Brown[37] found a gradual rise in the late afternoon and early evening, a gradual drop to a minimum at 4 A.M., and a return to the morning level in the systolic, similar but smaller changes in the diastolic, and rises after meals.

The median of 252 blood pressure determinations (Chart 3) during the waking hours of five consecutive and twenty-one alternate days over a period of seven weeks was 88 diastolic, 27 pulse pressure, and 116 systolic.

The median diastolic maintains a level of 86 from waking until after noon, rises sharply to a peak of 108 before midafternoon, and gradually declines to 85 toward midnight. The pulse pressure begins the day at 22, soon rises at first gradually and then more abruptly to a peak of 38 shortly past midmorning, recedes to 24 by noon, rises sharply to a peak of 37 in the early afternoon, and declines toward late afternoon to 27, which it maintains through the evening. The resulting systolic pressure starts at 108, rises to 125, recedes to 111, rises sharply to 130, and gradually declines to 112. The medians of the readings from these trends on successive hours through the day are 85 diastolic, 27 pulse pressure, and 114 systolic.

The course of the blood pressure is intimately associated with the routine of activity. The earlier rise in the pulse pressure and in the systolic pressure begins on rising and breakfasting, reaches its peak on arrival at the office, and then subsides. The later rise in the pulse pressure begins a little before lunch and reaches its peak subsequent to arrival at an afternoon clinic. The concomitant rise in the diastolic pressure begins slightly later and reaches its peak an hour later. The second peak of the systolic pressure is the resultant of these two rises. No after-dinner increase is sufficiently salient to influence the trends, but in the early evening hours points preponderate slightly above them. This represents the course of the blood pressure associated with the usual routine of

activity. When the routine was drastically transgressed blood pressure readings most often could not be taken.

But over week-ends, when professional activities were interrupted, the course of the blood pressure altered radically. The peaks were diminished or levelled. The diastolic level was 1 mm. lower through the morning, the afternoon peak one-third its week-day altitude, and the decline to the usual late evening pressure very gradual. The peaks and trough in the pulse pressure were obliterated and replaced by a slight upgrade from the morning to the evening level. The systolic pressure accordingly began near its usual level, made a slight rise during the morning, mounted to a broad flat peak of 5 mm. in the earlier afternoon, and receded but slightly to its late evening height.

TABLE III

SODIUM NITRITE IN NORMAL ARTERIAL TENSION

DOSAGE	SODIUM NITRITE EXCRETED		MAXIMUM BLOOD PRESSURE REDUCTION			
			SYSTOLIC		DIASTOLIC	
			ACTUAL	TREND	ACTUAL	TREND
gr.	mg.	per cent	per cent	per cent	per cent	per cent
0.5	0.0060	0.0185	3.8	5.4	0.0	0.0
1.0	0.0000	0.0000	9.8	7.7	0.0	0.0
1.5	0.0172	0.0176	15.2	9.0	6.4	0.2
2.0	0.0000	0.0000	7.9	10.0	0.0	0.7
2.5	0.0026	0.0016	11.4	10.8	2.7	2.8
	0.0296	0.0181	4.0		0.0	
3.0	0.0246	0.0126	19.1	11.3	18.2	7.2
	0.0717	0.0368	9.3		7.2	
3.5	0.0893	0.0393	13.3	11.9	11.4	8.4
	0.0000	0.0000	5.8		6.1	
4.0	0.0049	0.0019	16.0	12.3	10.4	8.7
	0.0822	0.0316	10.2		7.9	
4.5	0.0413	0.0141	11.3	12.6	6.4	8.8
	0.0736	0.0252	13.4		10.6	
5.0	0.0214	0.0066	8.8	12.8	9.9	8.9
	0.0886	0.0272	14.0		7.8	

During the week no distinctively high pressure days or low pressure days occurred. But days of stability in which the blood pressure followed its trends varied with days of instability in which it oscillated widely and erratically across the medians. Often the pulse pressure tended to adhere to its trend, even though the diastolic, and as a result the systolic, pressure fluctuated.

The scatter of the individual points is wide. It is greatest for the systolic, less for the diastolic, and least for the pulse pressure. But almost all points (respectively 83, 88, and 91 per cent) lie within plus or minus 10 mm. The large majority (57, 59, and 67 per cent) lie within plus or minus 5 mm. No increase in the scatter accompanies increase in pressure.[43]

The blood pressure is very labile even with normal arterial tension.[44] Considerable minute to minute fluctuation appeared in the successive readings in a determination. The range between maximum and minimum readings was from

1 to 24 mm. for the systolic and from 2 to 23 mm. for the diastolic. The mean was 9.6 systolic, and 9.7 diastolic. The modes were both 8, the medians 9. The lower quartile was 6 and the upper 12 systolic, and 7 and 12 diastolic. These distributions are almost identical for systolic and diastolic pressures.

BISMUTH SUBNITRATE IN NORMAL ARTERIAL TENSION

Bismuth subnitrate by mouth lowers the blood pressure in normal arterial tension (Charts 4, 5; Table I). The rapidity, intensity, and duration of the effect increase with dosage (Table II; Chart 6).

The effect begins as late as during the second hour with the smaller dosages, but in one-half hour with moderate and larger dosages.

With small and moderately large dosages, the maximum effect on the systolic pressure occurs at four hours, on the diastolic at five hours.

But with the bulkier dosages an earlier maximum occurs at three hours in the systolic, at three and one-half hours in the diastolic, a partial recession intervenes, and a later maximum follows at seven and one-half hours systolic, seven and three-fourth hours diastolic.

Beyond the principal maximum the effect decreases to a level it maintains until from the sixth hour with the smallest dosage to nine and three-fourth hours with moderate and larger dosages.

The effect ceases after from five and three-fourth hours for the smallest dosage to thirteen and one-half hours for the larger dosages in the systolic, and from six and one-half to twelve and one-fourth hours in the diastolic.

The principal maximum in the systolic increases from 15.4 mm. or 13.8 per cent with the smallest dosage, at a gradually increasing rate up to a large dosage, and beyond it at a gradually decreasing rate, approaching a limit of 29 mm. or 23 per cent with the bulkier dosages. In the diastolic it increases, from 8 mm. or 9.4 per cent, at a rapidly decreasing rate up to a moderately large dosage, and beyond it at a gradually increasing rate.

The primary maximum with the bulkier dosages is uniformly 19 mm. or 15.2 per cent in the systolic, 13 mm. or 14.7 per cent in the diastolic. These values are similar to those of the next lower dosage giving a single maximum. The second maximum increases in continuity with the primary maximum of lesser dosages.

The sustained effect increases in the systolic, from 4 mm. or 3.5 per cent with the smallest dosage, at a gradually decreasing rate up to a large dosage, and then at a uniform rate; in the diastolic it increases, from 4.4 mm. or 5.2 per cent with the smallest dosage giving it, over the same interval in a lesser degree, and then remains constant at 6 mm. or 7.1 per cent.

Thus the increase of the blood pressure lowering effect of bismuth subnitrate by mouth with dosage encounters several limits in normal arterial tension. The rapidity of onset reaches a temporal limit with small or very moderate dosage, the continuance of the sustained effect with moderate dosage, and the duration with moderately large dosage. Although their times are fixed, the dosage determines the occurrence of one or two maxima. The sustained diastolic effect

reaches an intensive limit with moderately large dosages, and the systolic maximum beyond the bulkiest dosage.

The lability range of successive readings during the intervals of blood pressure reduction was from 2 to 30 for the systolic, from 3 to 22 for the diastolic. The mean was 10.5 systolic, 9.5 diastolic. The modes were 8. The median was 10, the lower quartile 7, the upper 14, systolic, and 8, 7, and 12, diastolic. The systolic range was increased, the centering constants raised, and the interquartile interval extended. The diastolic range was slightly decreased but the centering constants were not significantly lowered.

CHEMISTRY AND PHARMACOLOGY

The basic bismuth nitrates arise from the hydrolysis of normal bismuth nitrate. The primary hydrolytic product is the bismuth oxynitrate monohydrate[45] which dissociates into hydrated bismuth oxide cations and nitrate ions, and into anions of the complex acid of the hydrate of undissociated bismuth oxynitrate and hydrogen ions. By combination and rearrangement these ions form a series of interconvertible intermediary and stable molecular and ionic aggregates of bismuth oxynitrate, bismuth hydroxide, water, and nitrate, the constitution of which depends on the temperature and concentration of the solution. Bismuth subnitrate is a mixture of these basic bismuth nitrates.

Bismuth subnitrate reacts incompletely with hydrochloric acid, in concentrations corresponding to normal or increased gastric acidity, at body temperature, to form the oxychloride and the soluble chloride.[46] After the ingestion of its basic salts, bismuth is accordingly absorbed in insignificant amounts, proportional to the degree of gastric acidity and their sojourn in the stomach, but irrespective of their duration in the intestines, and excreted by the kidneys,[47] salivary glands, cecum, and colon.*

Bismuth subnitrate is difficultly soluble in water. The nitrate in the basic bismuth salts has a different reaction capacity according to whether it is directly attached to bismuth or is attached to the aggregate as an ion which can be neutralized by dilute hydroxide.[45] Bismuth subnitrate in suspension in distilled water at body temperature furthermore sets nitric acid free immediately and in slowly increasing quantity by hydrolysis.[46]

After the ingestion of bismuth subnitrate nitrate is therefore available in appreciable concentration in the gastrointestinal tract.

The promptness of the onset of the blood pressure lowering effect suggests the possibility that nitrate absorbed under these conditions may be reduced in the tissues in sufficient quantity to produce an appreciable nitrite effect.

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*Free bismuth ions occur in suspensions of bismuth subnitrate,[46] and are therefore also available for absorption by the intestine.

Yet bismuth subnitrate has been administered over long periods of time in arterial hypertension without giving rise to any symptoms or signs of intoxication.[15-18]

Despite its long-established widespread and intensive use in gastrointestinal disorders, only two clinical instances of metallic poisoning have been reported.[72, 73]

Chronic, perhaps subclinical, poisoning however, may occur. Kidney lesions, consisting of calcification of necrosing tubular epithelium,[74] probably of long standing and produced by long-continued self-medication with bismuth compounds for gastrointestinal disease, are an occasional incidental autopsy finding (personal communication).

Bismuth can be absorbed from the intestinal tract either through the production of its chloride or through ionization. If the production of the chloride is the major factor, this could be obviated by administering bismuth subnitrate in enteric capsules, so as to minimize, if necessary, its toxic effects.

Stepanow[48] found nitrite in certain tissues of dogs and rabbits on ordinary diet but not after a nitrate-free diet. After nitrate-free diet these tissues contained nitrite upon intravenous injection of sodium nitrate. Grinding nitrite-negative tissue with nitrate yielded nitrite. Bernheim and Dixon[49] showed that liver substance, and the muscle substance of some species, reduces nitrate to nitrite.

The unabsorbed nitrate is subject to bacterial action. In a substrate of alkaline reaction and in the presence of a sufficient abundance of organic nutrient material, numerous bacteria, including B. coli communis, B. coli communior, B. acidi lactici, B. lactis aerogenes, and B. alkaligenes faecalis, actively convert potassium and other soluble nitrates into nitrites.[50] The completeness of the conversion and the rapidity with which it reaches its maximum increase with the dilution of the nitrate.[51] The reduction proceeds with the access as well as in the absence of oxygen. The presence of nitrate is without prejudice to the growth of the bacteria, and only on increasing its concentration is their development inhibited.[50]

Nitrate is reduced to nitrite in the alimentary canal. After massive poisoning with sodium nitrate Barth[52] found nitrite in the intestinal content of cattle. After instillation of nitrate isolated intestinal loops of dogs contained nitrite. After excessive dosages of sodium nitrate Binz and Gerlinger[53] demonstrated large amounts of nitrite in the small intestinal content of dogs and rabbits.

Bismuth subnitrate reacts like the soluble nitrates. In the presence of bismuth subnitrate cultures of B. coli produce nitrite.[8, 10, 11] The intestinal content of cats showing signs of nitrite poisoning after ingestion of bismuth subnitrate contains nitrite.[10]

Bismuth subnitrate maintains nitrate constantly present in the intestinal content in great dilution. In man the conditions favorable to the reduction of nitrate to nitrite obtain, and the requisite bacteria preponderate in the lower ileum and the colon. As the bismuth subnitrate reaches these parts, its free nitrate should accordingly be reduced, and the resulting nitrite absorbed. Nitrite does in fact arise from bismuth subnitrate in the large intestine.[2, 5-11] Nitrite is found in intestinal content containing bismuth subnitrate.[9, 11] Such nitrite is absorbed into the body fluids.[8, 9] Feces, with fewer viable, and more attenuated, bacteria than intestinal content, can still reduce bismuth subnitrate to nitrite.[8, 11]

The time relationships of the blood pressure lowering effect of bismuth subnitrate by mouth are affected by gastrointestinal motility. On the occasion when the bismuth subnitrate was taken the preceding evening and the effect lasted into the following day, and on the two occasions when it was taken in the early morning hours (Table I), the time of each phase of the blood pressure reduction, observed after an interval of several hours' sleep, was markedly delayed in comparison with when the drug was taken on rising and had the advantage of daytime peristalsis and a small aftercoming breakfast and luncheon (Chart 4).

The bismuth subnitrate and its products themselves influence intestinal motility. The times of both the initial appearance and the complete evacuation of bismuth feces increase with the dosage of bismuth subnitrate (Table I).

With the bulkier dosages of bismuth subnitrate a uniform primary maximum blood pressure reduction is, after an intervening recession, followed by a variable second maximum (Charts 4, 6; Table II). A certain mass of bismuth subnitrate, on reaching the site of nitrate reduction, may liberate such a quantity of nitrite, that peristalsis is retarded, and further supply delayed. As this subnitrate expends its nitrite the inhibition is relaxed. When peristalsis resumes the remaining bismuth subnitrate is brought up to contribute its nitrite.

With lesser dosages of bismuth subnitrate the maximum blood pressure reduction is followed by a recession to a relatively sustained level (Charts 4-6; Table II). The first arrival of bismuth subnitrate at the site of nitrate reduction may then liberate such a quantity of nitrite, that its continued advance is delayed, until an equilibrium is established between bismuth subnitrate supply and nitrite production.

The therapeutic benefits of bismuth subnitrate in diarrhea may be due less to its mechanical distribution over the mucosa than to its liberation of nitrite which depresses the muscularis.

Only during a small fraction of the time bismuth is present in the intestinal tract is the nitrite effect of its subnitrate exerted. The discontinuance of the effect is not due to exhaustion of the nitrate, for, if after the first blood pressure reduction, it happens that the equilibria among the intestinal flora are so deranged, that their biochemistry takes a turn toward putrefaction, a subsequent reduction ensues, at least equal to the original in duration and intensity (Table I; Charts 4, 5). The subsidence and cessation of the nitrite effect while nitrate is still available might be due either to the transport of the bismuth subnitrate to a part of the intestine unfavorable for its reduction to nitrite or to the further reduction of nitrite. In the presence of carbohydrate or the higher alcohols, the bacilli, which reduce nitrate to nitrite, carry its reduction beyond nitrite, themselves elaborating ammonia, while others liberate free nitrogen.[50] The entire fund of nitrate derived from bismuth subnitrate is finally either absorbed, or reduced to nitrite, which is either absorbed, or further reduced. Bismuth feces contains neither nitrite (Table I) nor nitrate to be reduced to nitrite.[11]

Bismuth subnitrate by mouth in large dosage, Mehrtens, Hanzlik, et al.[54] observed, occasionally produces with controlled fluid intake, a diuresis, which is not due to the absorption of bismuth in view of the nonexcretion of bismuth in the urine, but is due to nitrite action as indicated by coincident nitrite effects on the circulation. With free fluid intake the dosage of bismuth subnitrate does not perceptibly influence the urinary output.

Bismuth subnitrate by mouth causes a reduction of the blood chlorides due to the chloride diuresis of nitrate.[16]

Nitrite is not excreted in the urine after bismuth subnitrate by mouth (Table I).

But sodium nitrite is partly excreted as nitrite (Table III). The excretion is irregular and minute. Even with dosages well in excess of the therapeutic, the total excretion, which tends to increase with dosage, did not exceed 0.089 mg. or 0.039 per cent. The sodium nitrite content of the urine ranged from liminal

to 0.0035 mg. per c.c. Nitrite excretion was independent of urinary output, the diminution of which generally corresponded to the blood pressure reduction, for the smaller volumes of urine contained higher concentrations of nitrite. Although the intitial excretion of nitrite often coincided with the peak of the blood pressure reduction, increased rapidity of blood pressure reduction did not conduce to increased nitrite excretion, and with a given dosage the amount of nitrite excreted was not related to the extent of the blood pressure reduction. Thus nitrite excretion did not seem to depend on the rate of its absorption, nor on renal factors, but on its metabolism. "The nitrate absorbed is excreted in the urine as nitrate although some of it may remain unoxidized."[55]

The overwhelming destruction and minute excretion of nitrite in the urine during and after the acute influx of nitrite into the blood stream incident to the absorption of sodium nitrite precludes the possibility of its excretion during the gradual prolonged access of nitrite derived from enteric bismuth subnitrate in limited dosages.

The blood pressure lowering effect of sodium nitrite in normal arterial tension (Table III) was irregular but in the systolic the increase in the maximum tended to become continuously less with successive increases of the dosage, as in an exponential curve, approaching a limit of 13 per cent, and in the diastolic to become greater and then less, as in a sigmoid curve, approaching a limit of 9 per cent.

Bismuth subnitrate if completely converted into nitrite would be equivalent to less than 24.1 per cent its weight of sodium nitrite. No inference as to the actual degree of its conversion is possible because the nitrite effect depends on the relative rates of absorption and destruction. But moderate dosages of bismuth subnitrate by mouth maintain a blood pressure reduction comparable with the maximum reduction of therapeutic dosages of sodium nitrite.

Nitrite absorbed as such does not produce tolerance.[56]

DISCUSSION

Arterial hypertension is mediated by increased arteriolar resistance.[57, 58] Cardiac work is thereby increased.[58] As failure impends cardiac output and the velocity of blood flow decrease.[58-60]

The abnormal arteriolar resistance is largely functional and can be reduced by dilatation.[57] Sustained reduction of the arteriolar resistance should obviate the increased demand upon the heart, defer its late secondary effects on the circulation, and avoid the concomitant hypertrophy of the left ventricle[61] and the hastened development of arteriolosclerosis.[62]

Among "the pharmacologic and therapeutic characteristics essential to a substance that is to be beneficial in arterial hypertension" is that it "must decrease the arteriolar resistance without disturbing essentially the other fundamental normal characteristics of the circulation, such as the cardiac output, the velocity of the blood flow, or the circulating blood volume. The effect of the substance and the persistence of its action on the blood pressure must be such that by repeated administration of the drug marked fluctuations in the blood

pressure are not produced. Establishment of a permanent lower level should occur gradually. The normal vasomotor responses essential to normal bodily functions should not be affected.''[63]

Bismuth subnitrate by mouth effects a reduction of blood pressure. In intensity its effect is comparable to that of the other members of the nitrite group.[39] But the nitrite effect of the insoluble basic bismuth nitrates is more prolonged than even that of the higher alkyl nitrates.[38-40] The dosage can be manipulated to give the desired degree of blood pressure reduction. By repetition of the dosage at proper intervals a temporal summation of effect as in incomplete tetanus may be obtained. The blood pressure might by this means be maintained at a relatively constant reduced level. The nitrite action is exerted almost exclusively on the arterioles. Vasomotor responses, while moderated, remain intact.

CONCLUSIONS

1. Bismuth subnitrate by mouth produces prolonged and effective reduction of the blood pressure in normal arterial tension.

2. Bismuth subnitrate by mouth is available for the therapy of arterial hypertension.

3. Bismuth subnitrate by mouth merits adequately controlled application in arterial hypertension to definitely evaluate its therapeutic efficacy.

ADDENDUM

Blood Pressure Lowering Effect of Inorganic Nitrates by Mouth

Since the blood pressure lowering effect of bismuth subnitrate by mouth was investigated because of toxicologic[1-14] and therapeutic[15-18] rather than analytic considerations, the investigation was extended to other inorganic nitrates to determine the possible occurrence of similar effects.

Nitrate-reducing bacteria convert the nitrates of sodium, potassium, ammonium, and calcium to nitrite.[50] Sodium nitrate is reduced to nitrite in the animal intestine,[52, 53] within the organism,[48] and by certain tissues.[48, 49] Daily diuretic doses of ammonium nitrate occasionally produce cyanosis, methemoglobinemia, and nitrite symptoms in man.[64-67]

The procedure developed with the basic bismuth nitrates for determining the blood pressure lowering effect of drugs acting during an indefinite period was reapplied with the normal nitrates of such metals as are relatively nontoxic by mouth.

The control blood pressures taken on alternate days were separately plotted and smoothed for week days and for week-ends. The week day diastolic and systolic pressures from about 7 A.M. to after 1 P.M. traversed the same cycles as previously (Chart 3), but in the diastolic the forenoon pressure was 2 mm. lower and the afternoon peak 1 mm. higher, and in the systolic the pressure was from 3 to 6 mm. higher throughout. The week-end diastolic pressure maintained its previous morning level, but declined moderately during the afternoon, while the systolic began 2 mm. higher, gradually rose 4 mm. between 10 A.M. and 1 P.M., and as gradually returned to its original level during the later afternoon.

The ratios of the weight of the nitrate radicals to the total molecular weight of the selected nitrates (Table IV) and their reciprocals were calculated. The reciprocals multiplied by 1, 2, and 5 gm. fixed the amounts of comparable dosages of the several nitrates containing these equivalents of nitrate. Such dosages of nitrite-free chemically pure crystalline substances were weighed out to the nearer decigram (Table IV), freshly dissolved in small volumes of water, and gulped down, followed by repeated rinsings of the glass, to allay the taste in the mouth, and to avoid possible gastric irritation(which was manifested only with cerous nitrate which produced prolonged activation of the vomiting reflex), and after a short interval by a small breakfast.

The blood pressure responses were charted (Chart 7) and tabulated (Table IV).

The normal inorganic nitrates by mouth lower the blood pressure in normal arterial tension (Chart 7; Table IV). The effect is more intense and more consistent in the systolic than in the diastolic.

Nitrate equivalents of any of the alkalies, lithium, sodium, potassium, and ammonium, exert approximately equal effects. The nitrate equivalents of 2 gm. have only a slightly more intense and more prolonged effect than the nitrate equivalents of 1 gm. The nitrate equivalent of 5 gm., however, exerts a very much more acute and more intense effect.

Nitrate equivalents of the alkaline earths, calcium and strontium, also exert comparable effects. With the alkaline earths the effect is more sustained, though little more intense, with nitrate equivalents of 1 gm., than with the alkalies. But the effect becomes much more acute and twice as intense on doubling the nitrate equivalent.

With magnesium the nitrate equivalent of 1 gm. exerts an effect three times as intense as with the alkalies, or the alkaline earths.

With aluminium, which is 2.4 per cent hydrolized at 40° C.[68] this nitrate equivalent gives a more prolonged and somewhat more intense effect than with the alkalies or alkaline earths.

But with ferric nitrate, which is also hydrolyzed, the effect is slightly less than with the alkalies.

The relative intensity and duration of the blood pressure lowering effects of equivalents of different nitrates and of different equivalents of the same nitrate by mouth might be conditioned by the solubility of the nitrate, the permeability of the intestinal mucosa to its cation, and its influence on intestinal motility.

The normal metallic nitrates are all fairly soluble. Their relative solubility is indicated by the number of gm. anhydride per 100 gm. aqueous solution[68] interpolated for body temperature (Table IV). Such differences in solubility as exist between the nitrates tested do not show any distinct influence on the blood pressure lowering effect. Barium, dissolving only 11.8 gm. per 100 gm. solution,[68] is relatively much less soluble than the other nitrates. But since the dosage required to secure a comparable effect would be twice the equivalent of the stated probable lethal dose of its chloride,[69] it might prove inexpedient to ingest it during the course of an experiment.

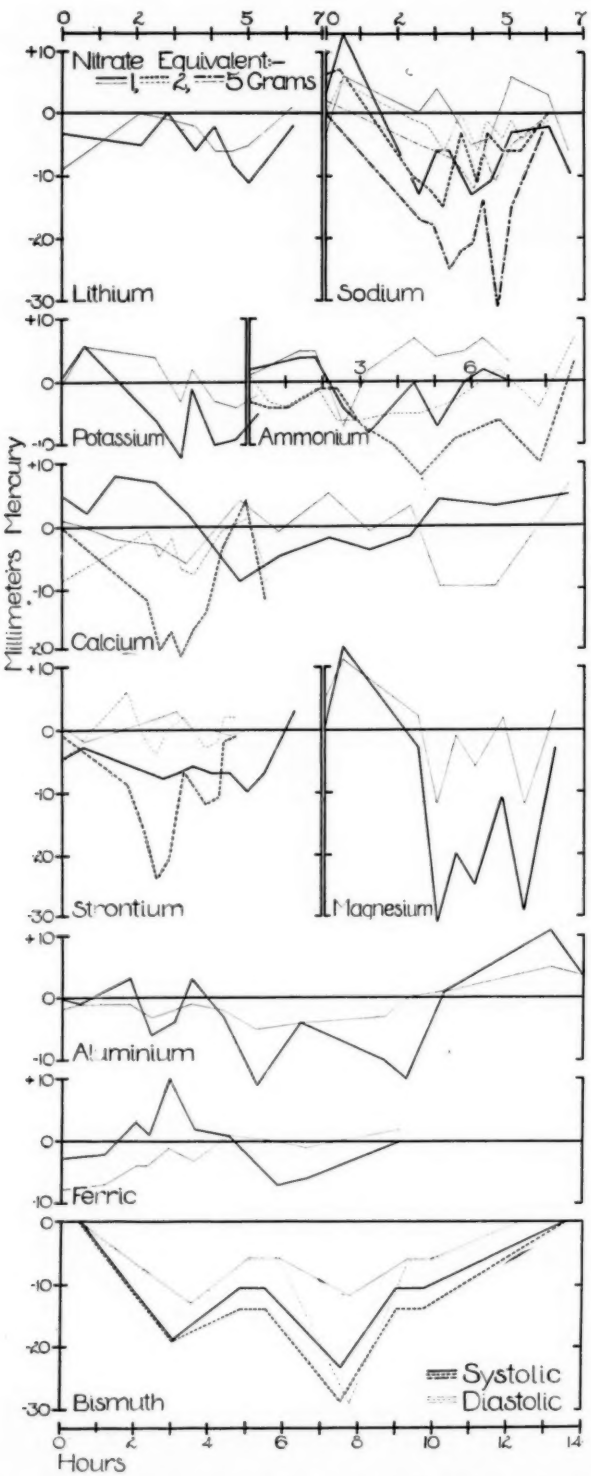

Chart 7.—Deviation of blood pressure from median after administration of inorganic nitrates by mouth in normal arterial tension.

TABLE IV

BLOOD PRESSURE LOWERING EFFECT OF INORGANIC NITRATES BY MOUTH: NORMAL ARTERIAL TENSION

NITRATE	FORMULA	MOLECULAR WEIGHT	NITRATE RATIO $\frac{xNO_3}{M^x(NO_3)x,yH_2O}$	SOLUBILITY gm. anhydride[1] / 100 gm. solution	CATION PERMEABILITY	DOSAGE gm.	SUBMEDIAN BLOOD PRESSURE											
							SYSTOLIC						DIASTOLIC					
							MEDIAN			MAXIMUM			MEDIAN			MAXIMUM		
							hr.	mm. Hg	per cent	hr.	mm. Hg	per cent	hr.	mm. Hg	per cent	hr.	mm. Hg	per cent
Lithium	$LiNO_3$	68.9	0.900	58.8	+++	1.1	3 - 6 1/2	7	5.9	5	11	9.3	2 1/12 - 6	5	6.0	4 1/3	6	7.2
Sodium	$NaNO_3$	85.0	0.729	50.5	+++	1.4	1 1/2 - 6	6	5.2	2 1/2	14	11.0	3 1/2 - 4 2/3	4½	5.6	3 11/12	5	6.0
						2.7	1 1/4 - 6	6	5.2	3 1/6	15	12.2	2 1/12 - 6	1½	1.8	3 1/3	6	7.2
						6.9	0 - 6	18	15.3	4 7/12	31	24.6	3/4 - 6	6	7.1	4	12	14.3
Potassium	KNO_3	101.1	0.613	36.4	+++	1.6	1 7/12 - 6	7½	6.4	3 1/6	12	9.6	2 11/12 - 5 5/6	3	3.6	4 2/3	4	4.8
Ammonium	NH_4NO_3	80.0	0.775	73.5	++++	1.3	2 1/2 - 5 3/4	5½	4.9	3 1/6	8	7.0		–5	–6.0			
						2.6	2 1/3 - 8 1/2	9½	8.5	4 7/12	15	13.2	1 5/6 - 6 1/6	5	6.0	2 2/3	6	7.2
Calcium	$Ca(NO_3)_2,4H_2O$	236.2	0.525	63.8	±	1.9	3 7/12 - 9 7/12	4	3.6	4 3/4	9	7.9	2/3 - 4 1/6	3	3.6	3 1/3	6	7.3
						3.8	0 - 4 7/12	17	14.4	3 1/6	21	17.2	2 1/4 - 4 1/2	5	6.0	3 1/2	8	9.5
Strontium	$Sr(NO_3)_2$	211.6	0.587	47.6	±	1.7	7/12 - 6	7	5.9	4 1/12	10	8.6		0	0.0			
						3.4	0 - 5	11	9.3	2 1/2	24	19.0		0	0.0			
Magnesium	$Mg(NO_3)_2,6H_2O$	256.4	0.482	45.2	±	2.1	2 1/4 - 6 1/3	22½	19.1	3 1/12	31	24.6	2 2/3 - 6 1/12	6	7.1	3 1/12	12	14.3
Aluminum	$2Al(NO_3)_3,9H_2O$	588.4	0.632	46.0	±	1.6	2 - 10 1/4	5	4.5	5 1/4	14	12.3	1 5/6 - 9 1/4	3	3.6	5 1/4	5	6.1
Cerous	$Ce(NO_3)_3,6H_2O$	434.4	0.428	soluble	±	2.3[2]												
Ferric	$Fe(NO_3)_3,9H_2O$	404.0	0.461	50.3	±	2.2	4 2/3 - 9 1/12	5½	4.9	5 5/6	7	6.1		4	4.8			
Basic Bismuth	$BiONO_3,H_2O$ (approximate)	304.0	0.204	insoluble	±	5.0[3]	1/2 - 13 1/2	10.4	9.1	3 7 1/2	19.0 23.4	15.2 19.1	1/2 - 12 1/4	6.0	7.1	3 1/2 7 3/4	13.0 11.8	14.7 13.0
						9.9	1/2 - 13 1/2	14.0	12.3	3 7 1/2	19.0 28.8	15.2 23.0	1/2 - 12 1/4	6.0	7.1	3 1/2 7 3/4	13.0 19.0	14.7 21.0

[1] *Landolt-Börnstein physikalisch-chemische Tabellen.*

[2] Hyperemesis experimentatoris.

[3] Table II: grains 75 and 150.

The permeability of the intestinal mucosa for the cation might influence the blood pressure lowering effect by affecting the duration of the ionic nitrate within the intestine. While the alkalies, especially ammonium, are all highly permeable,[67] the alkaline earths, and magnesium, aluminium,[70] and iron while penetrating the intestinal wall in minute amounts, are almost totally excluded[55, 69] (Table IV). The less permeable cations conduce to more prolonged and more intense effect, and to greater increase of the effect with increase in dosage. This tendency is offset by hydrolysis.

The ability to excite peristaltic waves, manifested by magnesium and the higher dosages of the other salts, is a more decisive factor. While impermeability of the cation may tend to detain the nitrate ion in the intestine, stimulation of peristalsis propels it directly to the site for nitrate reduction. Thus moderate catharsis increases the nitrite effect.

The nitrite effect of the normal inorganic nitrates is considerable. But it requires massive dosage. These nitrates are applicable only for diuresis. Very probably the renal action in nitrate diuresis[67] is in part a nitrite action.[54]

The nitrite effect of peroral nitrates depends on the reduction of nitrate in the intestinal canal. The production of nitrite is a race against the absorption of nitrate. The basic bismuth nitrates are preeminently fitted to give a prolonged and intense nitrite effect because of their relative insolubility, the practical nonabsorption of bismuth from the intestine, and their slight hydrolysis. The undissolved bismuth subnitrate mixed with chyme or feces preserves a latent store of nitrate which can be but slowly dissipated. As it enters into solution bismuth subnitrate maintains a constant low concentration of nascent nitrate ions through hydrolysis. Only a small part of this free nitrate comes in contact with the intestinal wall to be absorbed, but all is in intimate contact with and subject to the action of nitrate-reducing bacteria. Thus the nitrate in bismuth subnitrate is reserved in an insoluble compound, and, as sparingly liberated, relatively little absorbed as such, but for the most part converted into and absorbed as nitrite.

REFERENCES

1. Sailer, J., and Pancoast, H. K.: A Clinical Study of Gastroptosis With Special Reference to the Value of the Bismuth Skiagraph Shadow in Determining the Topography of the Gastrointestinal Tract, Univ. Pennsylvania Med. Bull. **19**: 130-140, 1906.
2. Rautenberg, E.: Methämoglobinvergiftung durch Sesamöl, Berlin. klin. Wchnschr. **43**: 1397-1398, 1906.
3. Prior, A.: Ein Fall von Wismut-Intoxication bei interner Darreichung von Magisterium Bismuti, München. med. Wchnschr. **54**: 1934-1935, 1907.
4. Zabel, E.: Zur Kasuistik und Symptomatologie der Vergiftung mit Bismutum subnitricum, Deutsche med. Wchnschr. **35**: 200-201, 1909.
5. Phillips, J.: Bismuth Poisoning and Nitrite Poisoning From Use of Bismuth Subnitrate, Cleveland Med. J. **16**: 419-428, 1917.
6. Hildebrand: Über die Methode durch Einbringen von Schattengebenden Flüssigkeiten Hohlorgane des Körpers sichtbar zu machen, Fortschr. a. d. Geb. d. Röntgenstrahlen. **11**: 96-99, 1907.
7. Benneke and Hoffmann, W.: Falles von Wismutvergiftung infolge stomachaler Verabreichung von Bismutum subnitricum bei einem 3 Wochen alten Kinde, München. med. Wchnschr. **53**: 945-946, 1906.
8. Böhme, A.: Über Nitritvergiftung nach interner Darreichung von Bismuthum subnitricum, Arch. f. exper. Path. u. Pharmakol. **57**: 441-453, 1907; München. med. Wchnschr. **55**: 89, 1908.

9. Meyer, E.: Ueber eine Vergiftung durch Bismuthum subnitricum, München. med. Wchnschr. **55**: 1905-1906, 1908; Vergiftung durch Bismuthum subnitricum und sein Ersatz durch Bismuthum carbonicum, Therap. Monatsh. **22**: 388-390, 1908.
10. Nowak, J., and Gütig, C.: Nitritvergiftung durch Bismutum subnitricum, Berlin. klin. Wchnschr. **45**: 1764-1768, 1908.
11. Zadek, J.: Ueber die Ursachen der Nitritvergiftung durch Bismutum subnitricum, Ztschr. f. exper. Path. u. Therap. **15**: 498-516, 1914.
12. Beck, E. G.: Toxic Effects From Bismuth Subnitrate, With Reports of Cases to Date, J. A. M. A. **52**: 14-18, 1909.
13. Zollinger, F.: Experimentell-klinische Beiträge zur Frage der Wismutvergiftung, Beitr. z. klin. Chir. **77**: 268-293, 1912.
14. Mayer, L., and Baehr, G.: Bismuth Poisoning, A Clinical and Pathological Report, Surg. Gynec. Obst. **15**: 309-322, 1912.
15. Stieglitz, E. J.: Bismuth Subnitrate in the Therapy of Hypertension, J. Pharmacol. & Exper. Therap. **32**: 23-35, 1927.
16. Stieglitz, E. J.: The Pharmacodynamics and Value of Bismuth Subnitrate in Hypertension, J. Pharmacol. & Exper. Therap. **34**: 407-423, 1928.
17. Stieglitz, E. J.: Bismuth Subnitrate in the Treatment of Arterial Hypertension, J. A. M. A. **95**: 842-846, 1930.
18. Stieglitz, E. J.: Arterial Hypertension, New York, 1930, Hoeber, 119-124, 136-141.
19. Ayman, D.: An Evaluation of Therapeutic Results in Essential Hypertension: I. The Interpretation of Symptomatic Relief, J. A. M. A. **95**: 246-249, 1930; II. The Interpretation of Blood Pressure Reductions, ibid. **96**: 2091-2094, 1931.
20. Ayman, D.: Potassium Thiocyanate in the Treatment of Essential Hypertension: Its Impracticability, J. A. M. A. **96**: 1852-1857, 1931.
21. Barksdale, I. S.: Studies on the Blood Pressure Lowering Principle in the Seed of Watermelon (Cucurbita Citrullus), Am. J. M. Sc. **171**: 111-123, 1926.
22. Wilkinson, G. R.: Further Studies on the Blood Pressure Lowering Effect of Cucurbocitrin in Man, J. South Carolina M. A. **23**: 366-369, 1927.
23. Althausen, T. L., and Kerr, W. J.: Watermelon-Seed Extract in the Treatment of Hypertension, Am. J. M. Sc. **178**: 470-489, 1929.
24. Report of the Council on Pharmacy and Chemistry, Citrin Not Acceptable for New and Nonofficial Remedies, J. A. M. A. **94**: 1067, 1930.
25. Barksdale, I. S.: A Review of Literature on Therapeutic Value of Citrin (Cucurbocitrin) in Vascular Hypertension, Med. J. & Rec. **133**: 139-141, 1931.
26. Seward, B. P.: The Extract of Watermelon Seed in Treatment of Hypertension, Virginia Med. Month. **57**: 788-795, 1931.
27. Gargil, S. L., and Rudy, A.: The Value of Cucurbocitrin in the Treatment of Arterial Hypertension, Am. J. M. Sc. **181**: 639-648, 1931.
28. Agatson, S. A.: Changes in the Fundus Oculi as a Definite Index to Arterial Disease, Arch. Int. Med. **42**: 455-466, 1928
29. Friedenwald, H., and Friedenwald, J. S.: The Retinal Blood Vessels in Hypertension and Arteriosclerosis, Bull. Johns Hopkins Hosp. **45**: 232-246, 1929.
30. Brown, G. E.: Daily and Monthly Rhythm in the Blood Pressure of a Man With Hypertension: A Three-Year Study, Ann. Int. Med. **3**: 1177-1189, 1930.
31. Mosenthal, H. O., and Short, J. J.: The Spontaneous Variability of Blood Pressure, Am. J. M. Sc. **165**: 531-550, 1923.
32. Sigler, L. H.: Spontaneous Non-Rhythmic Variations in the Blood Pressure, Am. J. M. Sc. **177**: 494-506, 1929.
33. Ayman, D.: Normal Blood Pressure in Essential Hypertension, J. A. M. A. **94**: 1214-1218, 1930; Essential Hypertension; The Diastolic Blood Pressure: Its Variability, Arch. Int. Med. **48**: 89-97, 1931.
34. Sigler, L. H.: Periodic Variability of Some Forms of Arterial Hypertension, Am. J. M. Sc. **172**: 543-548, 1926.
35. Stieglitz, E. J.: Arterial Hypertension: Evaluation of the Prognosis, Arch. Int. Med. **46**: 227-335, 1930.
36. Weysse, A. W., and Lutz, B. R.: Diurnal Variations in Arterial Blood Pressure, Am. J. Physiol. **37**: 330-347, 1915.
37. Mueller, S. C., and Brown, G. E.: Hourly Rhythms in Blood Pressure in Persons With Normal and Elevated Pressures, Ann. Int. Med. **3**: 1190-1200, 1930.
38. Matthew, E.: Vaso-Dilators in High Blood Pressure, Quart. J. Med. **2**: 261-278, 1909.
39. Wallace, G. B., and Ringer, A. I.: The Lowering of Blood Pressure by the Nitrite Group, J. A. M. A. **53**: 1629-1630, 1909.
40. Lawrence, C. H., Jr.: The Effect of Pressure-Lowering Drugs and Therapeutic Measures on Systolic and Diastolic Pressure in Man, Arch. Int. Med. **9**: 409-419, 1912.
41. Sewall, H.: On the Clinical Investigation of Postural Changes in the Blood Pressure, and the Secondary Waves of Arterial Blood Pressure, Am. J. M. Sc. **158**: 786-816, 1919.

42. Standard Methods for the Examination of Water and Sewage, ed. 5, New York, 1923, American Public Health Service, 18-19.
43. Diehl, H. S.: The Variability of Blood Pressure: Morning and Evening Studies, Arch. Int. Med. **43**: 834-845, 1929.
44. Hare, D. C.: The Variation of Blood Pressure Readings Studied Under Uniform Conditions, Proc. Roy Soc. Med. **19** (i): 36-38, 1926.
45. Hepner, B.: Untersuchungen über Wismutverbindungen: II. Über die Konstitution der Basischen Wismutnitrate, Arch d. Pharm. u. Ber. d. Deutsch. pharm. Gesellsch. **264** (Jahrg. 36): 55-65, 1926.
46. Traffehn, W.: Beitrag zur Hydrolyse von Wismutverbindungen, Inaug. Diss., Friedrich-Wilhelms Univ., Berlin, 1927, 3, 5-7, 10, 17-18. Thoms, H., and Traffehn, W.: Über die Hydrolyse von Wismutverbindungen, Arb. a. d. pharm. Inst. d. Univ. Berlin **13**: 492-503, 1927.
Stingel, J. L.: The Fixation of Sulphide by Basic Bismuth Compounds, Am. J. Pharmacol. **83**: 412-413, 1911.
47. Dorner, S., and Weingärtner: Über Wismut-ausscheidung im Urin nach Wismutmahlzeit, Deutsches Arch. f. klin. Med. **98**: 258-266, 1909.
48. Stepanow, A.: Über die Zersetzung des Jodkaliums im Organismus durch Nitrite, Arch. f. exper. Path. u. Pharmakol. **47**: 411-416, 1902.
49. Bernheim, F., and Dixon, M.: The Reduction of Nitrates in Animal Tissues, Biochem. J. **22**: 125-134, 1928.
50. Maasen, A.: Die Zersetzung der Nitrate und der Nitrite durch die Bakterien, Arb. a. d. kaiserl. Gesundheitsamte **18**: 21-77, 1902.
51. Peltz, E.: Über Nitritbildung durch Bakterien, Centralbl. f. Bakt., 1 te Abt., Orig. **57**: 1-16, 1910.
52. Barth, A.: Toxikologische Untersuchungen über Chilisalpeter, Inaug. Diss., Pharmakol. Inst., Bonn, 1879, cited by Binz and Gerlinger.
53. Binz, C., and Gerlinger, P.: Die Reduktion des Natriumnitrats im Tierkörper, Arch. internat. de Pharmacodyn. et de Therap. **9**: 441-450, 1901.
54. Mehrtens, H. G., Hanzlik, P. J., Marshall, D. C., and Brown, N. S.: Bismuth as a Diuretic, J. A. M. A. **91**: 223-225, 1928.
55. Cushny, A. R.: A Textbook of Pharmacology and Therapeutics, ed. 9, revised by C. W. Edmunds and J. A. Gunn, Philadelphia, 1928, Lea & Febiger, 435.
56. Crandal, L. A., Jr., Leake, C. D., Loevenhart, A. S., and Muehlberger, C. W.: Acquired Tolerance to and Cross Tolerance Between the Nitrous and Nitric Acid Esters and Sodium Nitrite in Man, J. Pharmacol. & Exper. Therap. **41**: 103-119, 1931.
57. Ellis, L. B., and Weiss, S.: The Measurement of Capillary Pressure Under Natural Conditions and After Arteriolar Dilatation in Normal Subjects and in Patients With Arterial Hypertension and With Arteriosclerosis, J. Clin. Invest. **8**: 47-67, 1929.
58. Weiss, S., and Ellis, L. B.: The Quantitative Aspects and Dynamics of the Circulatory Mechanism in Arterial Hypertension, Am. Heart J. **5**: 448-468, 1930.
59. Blumgart, H. L., and Weiss, S.: Studies on the Velocity of Blood Flow: IV. The Velocity of Blood Flow and Its Relation to Other Aspects of the Circulation in Patients With Arteriosclerosis and in Patients With Arterial Hypertension, J. Clin. Invest. **4**: 173-197, 1927.
60. Blumgart, H. L., and Weiss, S.: Clinical Studies on the Velocity of Blood Flow: IX. The Pulmonary Circulation Time, the Velocity of Venous Blood Flow Through the Heart, and Related Aspects of the Circulation of Patients With Cardiovascular Disease, J. Clin. Invest. **5**: 343-377, 1928.
61. Fahr, G.: Hypertension Heart, Am. J. M. Sc. **175**: 453-472, 1928.
62. Fishberg, A. M.: Anatomic Findings in Essential Hypertension, Arch. Int. Med. **35**: 650-668, 1925.
63. Weiss, S., and Ellis, L. B.: The Rational Treatment of Arterial Hypertension, J. A. M. A. **95**: 846-850, 1930.
64. Christian, H. A.: Discussion, Trans. Assn. Amer. Physicians **43**: 290, 1928.
65. Barker, M. H., and O'Hare, J. P.: The Use of Salyrgan in Edema, J. A. M. A. **91**: 2060-2064, 1928.
66. Eusterman, G. B., and Keith, N. M.: Transient Methemoglobinemia Following Administration of Ammonium Nitrate, Med. Clin. North America **12**: 1489-1496, 1929.
67. Keith, N. M., Whelan, M., and Banniek, E. G.: The Action and Excretion of Nitrates, Arch. Int. Med. **46**: 797-832, 1930.
68. Landolt-Börnstein physikalisch-chemische Tabellen, ed. 5, edited by W. A. Roth and K. Scheel, Berlin: Springer, 1923, I, 632-688, III, 1165-1175; 1931, Zweiter Ergänzungsband, Erster Teil, 343-369.
69. Sollmann, T.: A Manual of Pharmacology and Its Application to Therapeutics and Toxicology, ed. 3, Philadelphia, 1926, Saunders, 534-535.

70. Underhill, F. P., and Peterman, F. I.: Studies in the Metabolism of Aluminium: III. Absorption and Excretion of Aluminium in Normal Man, Am. J. Physiol. **90**: 40-51, 1929.
71. Ayman, D.: Bismuth Subnitrate in the Treatment of Arteriolar (Essential) Hypertension, J. A. M. A. **98**: 545-548, 1932.
72. Cohn, M.: Kurze Mittheilung zur innerlichen Verabreichung von Bismuth. subnitric., Therap. Monatsh. **10**: 466-467, 1896.
73. Resnik, W. H.: Bismuth Poisoning Following Oral Administration of Bismuth Subnitrate, Bull. Johns Hopkins Hosp. **38**: 333-338, 1926.
74. Weller, C. V.: Calcification of the Kidneys in Bismuth Poisoning, Sc. Proc. Twenty-Ninth Annual Meeting of American Association of Pathologists and Bacteriologists, Am. J. Path. **5**: 541, 1929.

THE ORGANIC FRACTION OF URINARY PHOSPHORUS*

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IN A previous paper by one of us[1] the question was raised concerning the amount and significance of the "organic" or "unoxidized" fraction of the phosphorus of the urine. In this present report we have attempted to establish normal values for the twenty-four-hour elimination of organic phosphorus, and to study variations from the normal in a limited number of pathologic conditions.

By using twenty-four-hour collections, we have been able to secure sufficient amounts of urine to use a direct method of analysis, in place of the indirect method described in the previous paper. The method which we have used and will describe is based upon the one proposed by Youngburg and Pucher[2] and is the same in principle. We have, however, radically modified the mechanics of the procedure, believing that our modifications have not only slightly increased the accuracy of the method, but also simplified it and made it less time-consuming.

Procedure.—Prepare magnesia mixture by dissolving 55 gm. of crystalline magnesium chloride and 70 gm. of ammonium chloride in water, adding 88 c.c. of concentrated ammonia water (sp. gr. 0.90) and diluting to one liter. Let stand three days and filter before using. To 100 c.c. urine add 30 c.c. magnesia mixture. Let stand six to eighteen hours, filter through an ashless paper, using a dry funnel and receiver. Transfer 65 c.c. of the filtrate (equivalent to 50 c.c. urine) to a porcelain evaporating dish. Add 15 gm. crystalline magnesium nitrate and evaporate to dryness. Ignite directly over a free flame until the residue is white, and brown fumes cease to be given off. Cool, dissolve the residue in 15 to 20 per cent hydrochloric acid and again evaporate to dryness on a steam-bath. Transfer the residue to a 100 c.c. volumetric flask, using about 60 c.c. of water for the transfer. To another 100 c.c. volumetric flask add 5 c.c. standard phosphate solution (0.3509 gm. pure monopotassium phosphate per liter made up in approximately tenth normal sulphuric acid: 5 c.c. equals 0.4 mg. P) and 60 c.c. water. To both flasks add 10 c.c. Molybdate I (2.5 per cent ammonium molybdate in 5N sulphuric acid) and 4 c.c. of the Fiske-Subbarow reducing agent (1-2-4 aminonaphtholsulphonic acid made up according to the directions given by Fiske and Subbarow).[3] Make up to volume and after ten minutes read in the Duboscq colorimeter. The standard is set at 20 and the milligrams of phosphorus in the organic fraction per c.c. of urine is obtained by dividing 8 by the reading of the unknown solution and dividing by 50.

Blank determinations were made on the various reagents, separately and combined, and no amounts of phosphorus sufficient to give a color with the Fiske-Subbarow reagents were found.

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Five cubic centimeters of the standard phosphate solution was treated with magnesium nitrate and ignited, dissolved and read against an untreated 5 c.c. of standard phosphate solution with no gain or loss. The rather large content of the unknown in magnesium salts does not interfere with the color development.

Normal Values.—Measurements of the twenty-four-hour output of inorganic and of organic phosphorus were made on a group of young adults, chiefly laboratory workers, all of whom were free from any signs of metabolic disease.

The results are summarized in Table I (per twenty-four hours).

TABLE I

SUBJECT	TESTS MADE	MG. ORGANIC P			% OF TOTAL P		
		MAX.	MIN.	MEAN	MAX.	MIN.	MEAN
BW male	4	10	8	9	1.3	0.8	1.2
MD male	4	11	10	11	1.7	1.1	1.4
Both males	8	11	8	10	1.7	0.8	1.3
EW female	22	11	6	8	1.9	1.0	1.3
MH female	8	13	8	9	2.1	0.9	1.2
DB female	8	11	7	9	2.0	0.8	1.3
EG female	8	12	6	9	1.4	0.9	1.2
All females	46	13	6	9	2.1	0.8	1.3
All normals	54	13	6	9	2.1	0.8	1.3

In the female subjects, the series of tests in each case covered a period corresponding to a menstrual cycle. No periodicity in the elimination of either organic or inorganic phosphorus was observed.

Hospital Group.—This group consisted of 57 unselected cases from the endocrine diagnostic service of the Evans Memorial. The variations here were somewhat greater.

Organic phosphorus (mg. per 24 hours)	Maximum	17
	Minimum	2
	Mean	8
(per cent of total P)	Maximum	2.8
	Minimum	0.6
	Mean	1.5

Only two of this group had organic phosphorus outputs exceeding the maximum of the normal group, while six were below the minimum. There being no marked correlation between the diagnosis and the organic phosphorus output in this group, we feel that it would be idle to speculate on possible causes of high and low values. Certain diseases appeared with sufficient frequency in this group, however, to enable us to decide that they were quite without effect on the organic phosphorus elimination: among these may be listed thyroid failure, pituitary hypo- and dysfunction, arthritis, pulmonary tuberculosis (early), and chronic infections of tonsils and nasal sinuses.

Leucemia.—Eighteen years ago Symmers[4] observed an increase in organic phosphorus in the urine of a case of lymphatic leucemia. As far as we know, this investigation has not been carried any further, and we felt that it was of interest to measure the organic phosphorus output in leucemia. By the courtesy of physicians at the Boston City Hospital and the Massachusetts General Hospital,

we were able to obtain urines from three cases of leucemia, representing the three recognized types. In two of these cases we were able to make repeated observations (Table II).

TABLE II

	ORGANIC PHOSPHORUS	
	MG. PER 24 HOURS	% OF TOTAL P
Case B. Lymphatic leucemia	13	5.0
Case D. Myelogenous leucemia (chronic)	20	1.4
	18	1.2
	23	1.7
	13	2.4
	12	2.3
	14	2.3
Case W. Monocytic leucemia (acute)	14	1.7
	17	2.2
	14	1.6
	16	2.1
	14	2.2
	8	1.1

It will be noted that these values are definitely high, and several of them are higher than the maxima of the other series by over 30 per cent. While the magnitude of these observed changes do not correspond exactly with those observed by Symmers, his observation seems to be definitely confirmed.

SUMMARY

1. An improved direct method for the quantitative determination of organic phosphorus in the urine is described.

2. The normal output of organic phosphorus per twenty-four hours is from 6 to 13 mg., with a mean value of 9 mg.

3. In leucemia the output is high, values up to 23 mg. being observed.

REFERENCES

1. Walker, B. S.: Normal Relationships of Blood and Urine Phosphorus, J. LAB. & CLIN. MED. **17**: 347-53, 1932.
2. Youngburg, G. E., and Pucher, G. W.: Analytical Methods and Observations on the Organic Phosphorus of the Urine, J. Biol. Chem. **62**: 31-44, 1924.
3. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**: 375-400, 1925.
4. Symmers, D.: A Contribution to the Knowledge of the Excretion of Organic Phosphorus in the Urine in Certain Pathological Conditions, J. Path. & Bact. **10**: 159-72, 1904.

COLLOIDAL GOLD TEST FOR POLIOMYELITIS IMMUNE BODIES IN BLOOD SERUM*†

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IN THE study and treatment of poliomyelitis it would be desirable to have a reliable and rapid method for detecting and measuring specific antibodies and the therapeutic value of serums from human and animal sources. If this method were also applicable to the study of possible carriers and susceptibility to the disease, to the evaluation of natural and acquired immunity, and of prognosis, such a laboratory procedure might fill a recognized need.

Current procedures that require numerous monkeys for virus neutralization tests are not only costly but demand highly specialized and uncommonly difficult technic. This method in the hands of the most skillful workers is not without drawbacks and is always time consuming. Where the time element may be important, this alone is a cogent argument for a procedure that does not require weeks for definite results.

The proposed method should prove valuable for reasons that have been enumerated and particularly in selecting serums from convalescent poliomyelitis patients and from the large group of normal adults that are now known to have specific serum antibodies for poliomyelitis.

Technic of Method.—The test to be described depends upon the well-known but perhaps little understood mechanism of the action of serum colloids in the presence of a colloidal gold sol and an electrolyte, in this instance a solution of 0.4 per cent sodium chloride. The delicacy of this test is enhanced by the addition of cholesterol to the sodium chloride, a procedure that intensifies the flocculation effect. The colloidal gold sol is prepared according to our method which has yielded a constantly uniform and dependable reagent.[1] The ingredients used in its preparation must be of the highest chemical purity.

Scrupulously clean glassware and syringes must be used throughout. The syringe may be rinsed with sterile distilled water prior to withdrawing the blood samples. Salt solution, citrate mixtures, or any other chemicals or preservatives must be rigidly excluded, and accurately calibrated Kahn pipettes only are to be used.

The specimens of serum, free from preservatives, are inactivated in a water-bath at 56° C. for one-half hour. Serum dilutions are made with fresh doubly distilled water and a series of ten narrow test tubes (1 by 13 cm.), *chemically clean*, is set up containing in all but the first two tubes 0.1 c.c. of serum or its dilution as follows:

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TUBE NO.	SERUM	DIST. H_2O	DILUTION	SERUM CONTENT
1	0.2 c.c.	—	—	0.2 c.c.
2	0.15	—	—	0.15
3	0.1	—	—	0.10
4	0.1	0.1	1-2	0.05
5	0.1 No. 4	0.1	1-4	0.025
6	0.05	0.25 Discard 0.2 c.c. of mixture	1-6	0.016
7	0.1 No. 5	0.1	1-8	0.012
8	0.05	0.45 Discard 0.3 c.c.	1-10	0.01
9	0.1 No. 8	0.1 Discard 0.1 c.c.	1-20	0.005
10	0.1 No. 7	0.3 Discard 0.3 c.c.	1-32	0.003

To all tubes are added 0.75 c.c. cholesterinized NaCl (0.4 per cent)* and 1 c.c. Colloidal Gold Sol, in the order named. The Gold Sol** should be recently prepared, stabilized by three or four days of storage, and not more than four weeks old, and controlled in two additional tubes as follows: (1) NaCl (0.4 per cent) 0.75 c.c. and 1 c.c. Colloidal Gold should give complete precipitate in three hours. (2) NaCl (0.4 per cent), 0.75 c.c. and 2 c.c. Colloidal Gold should show no precipitate in twenty-four hours or longer. All tests with serum and each new lot of reagent are checked in this manner (Fig. 1).

Depending upon the content of antibodies, tubes will show the following reaction that is indicated by a "curve" or series of numbers, as follows:

Key:
1. Complete precipitation; Rose ppt. Supernatant fluid colorless
2. Complete precipitation; Rose ppt. Supernatant fluid pale salmon
3. Complete precipitation; Rose ppt. Supernatant fluid salmon
4. Partial precipitation; Rose ppt. Supernatant fluid pale rose
5. Partial precipitation; Rose ppt. Supernatant fluid rose

Negative
6. No precipitation; — — Supernatant fluid pale rose
7. No precipitation; — — Supernatant fluid rose (same as neg. control)

*The NaCl solution for the test is prepared as follows: 1 gm. pure Cholesterol is added to 1 liter of 0.4 per cent sodium chloride, shaken three times a day for one week, and filtered.

**The Colloidal Gold Sol is prepared as follows:

A two-liter capacity *Florence Flask* should be used when one liter of reagent is desired and a one-liter flask for the preparation of 500 c.c. of the reagent. Erlenmeyer flasks are not satisfactory and should not be used.

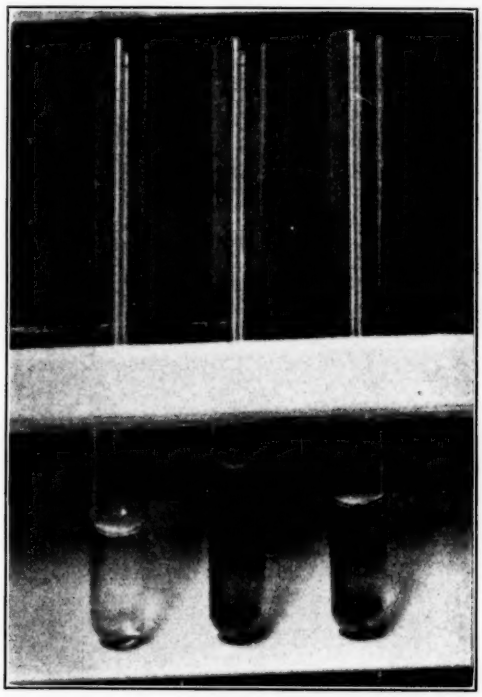
1,000 c.c. double distilled water,
8 c.c. 2 per cent potassium carbonate (Merck) (S. M.).
(Freshly prepared or not more than two weeks old.)
1 c.c. 1 per cent oxalic acid (Merck).

Particular care is necessary in heating the solution and attention is directed to the following details. The flask is placed on a piece of wire gauze having an asbestos center with a diameter of from 3¼ to 3½ inches. The Bunsen burner gas flame is so adjusted as to cover the asbestos disc completely but not to extend beyond its circumference. It will be noted that the hottest part of the flame covers the entire disc with the exception of a central circular area about an inch or more in diameter. This arrangement insures correct heating and the formation of large bubbles. Heat the flask on the wire gauze rotating the flask frequently until the *first large bubble* appears. This usually occurs after fifteen minutes. Remove flask, rotate contents once and while the fluid is in motion add 12 c.c. of 1 per cent Gold chloride (Merck). Replace on flame and heat until five large bubbles appear. †(Remove flask, rotate contents once and slowly add 5 drops (0.22 c.c.) of formaldehyde 40 per cent (C. P.) (Merck or Baker) while the fluid is still in motion. Rotate flask steadily until typical clear ruby color appears.) Promptly cover mouth of flask with a clean towel folded in several layers and moist with distilled water. Let stand undisturbed until cool.

Thermometric temperature control has proved valueless in the preparation of colloidal gold. The method described is simple and reliable. The reagent is allowed to stabilize for three or four days before use and is stored in the dark at a temperature of from 20 to 24° C. Suitable containers can be prepared by applying a coat of black lacquer to the exterior surface of the chemically clean glass stoppered bottles.

†At this point the technic has been modified with more satisfactory results, as follows:

Remove from flame and add 0.4 c.c. of a 1 to 10 dilution of 40 per cent formaldehyde "Mercks C. P." Shake until the ruby red color that appears in two to three minutes begins to take on an eosin tinge.



A *B* *C*

Fig. 1.—Control test of sensitivity of colloidal gold (24-hour reading). *A*, One cubic centimeter of colloidal gold completely precipitated in three hours in presence of 0.75 c.c. cholesterinized 0.4 per cent sodium chloride solution. *B*, Two cubic centimeters colloidal gold showing no change in presence of 0.75 c.c. cholesterinized 0.4 per cent sodium chloride solution, within or after twenty-four hours. *C*, Stock solution of colloidal gold without addition of cholesterinized sodium chloride solution.

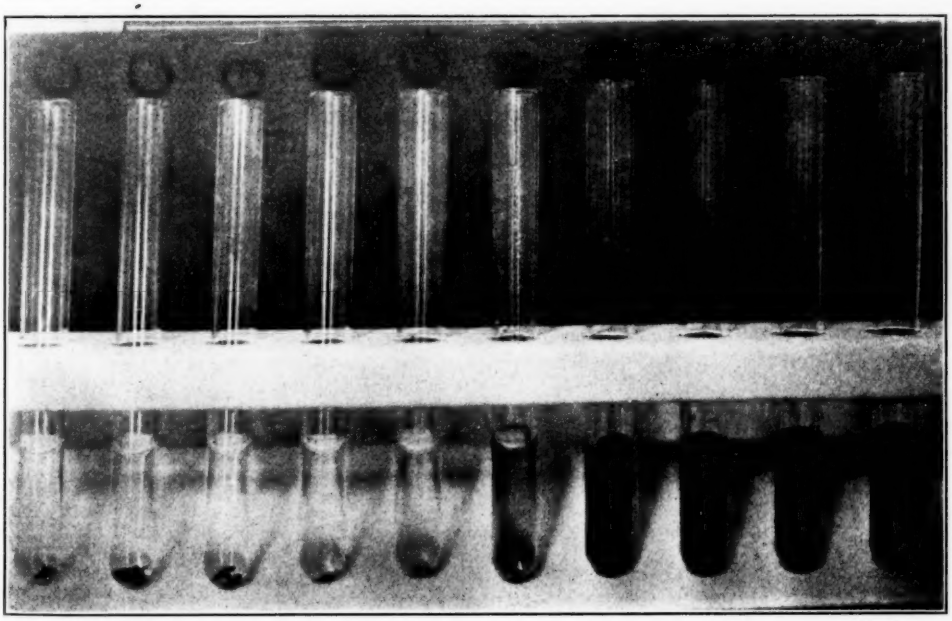


Fig. 2.—Positive colloidal gold test in the presence of normal adult serum having virucidal properties as shown by monkey inoculation. The reaction reads: 1111127777.

Tubes should be kept at room temperature (20° to 25° C.) and readings can be made after three or four hours for the earliest discernible changes. Final results are read after eighteen to twenty-four hours.

A strongly positive serum with high antibody content, for example, is indicated thus: 1111111122 (or 1111122333). A negative serum will read: 7777777777 (or 6666666666) (Figs. 2-4).

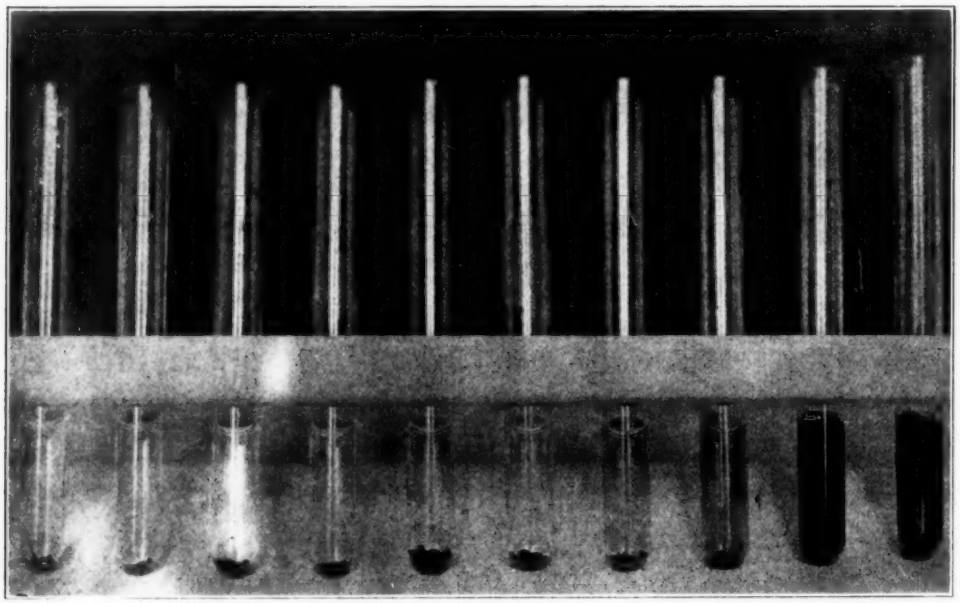


Fig. 3.—Positive colloidal gold test with serum from a convalescent poliomyelitis patient. The reaction reads: 1111111277.

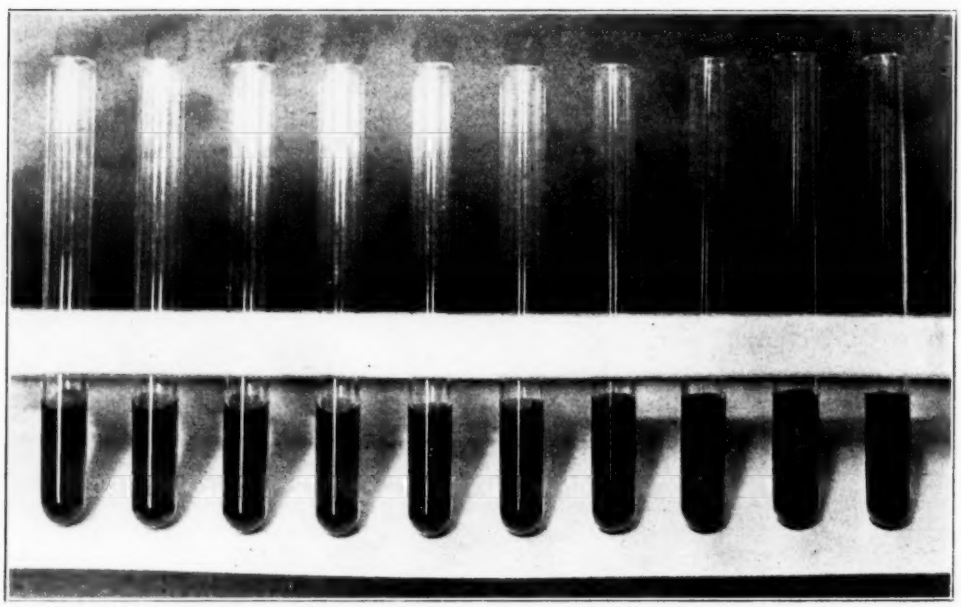


Fig. 4.—Colloidal gold test showing no change in the presence of a serum negative for poliomyelitis antibodies. The reaction reads: 7777777777.

TABLE I

INCIDENCE OF POSITIVE AND NEGATIVE TESTS FOR ANTIBODIES IN DIFFERENT AGE GROUPS

Adults (Age 25-50)

NUMBER	NEGATIVE	PER CENT	POSITIVE						TOTAL POSITIVE	PER CENT
			WEAK	PER CENT	MODERATELY STRONG	PER CENT	STRONG	PER CENT		
100	36**	36	14	14	20	20	30	30	64	64

Children (Age 6 Weeks-19 Years)

AGE	NUMBER	NEGATIVE	PER CENT	POSITIVE						TOTAL POSITIVE	PER CENT
				WEAK	PER CENT	MODERATELY STRONG	PER CENT	STRONG	PER CENT		
Under 1 yr. (6 wk., 13 wk., 7 mo.)	4	4	100.0	0	0	0	0	0	0	0	0
1 - 4 years	19	19	100.0	0	0	0	0	0	0	0	0
5 - 8 years	37	32	86.5	3	8.2	2	5.3	0	0	5	13.5
Total	60	55	91.7	3	5.0	2	3.3	0	0	5	8.3
9 - 14 years	35	25	71.4	2	5.7	5	14.3	3	8.6	10	28.6
15 - 19 years	41	31	75.6	2	4.9	3	7.3	5	12.2	10	24.4
Total	76	56	73.4	4	5.3	8	10.6	8	10.6	20	26.6
Total	136	111*	81.6	7	5.3	10	7.6	8	6.0	25	18.4

*This group included diphtheria immune (Schick negative) 94, typhoid immunity (strongly positive Widal) 5, and pneumonia immune serums, 3.
**This group included Schick negative as well as Widal positive.

MATERIAL STUDIED

Serums were obtained from normal and convalescent children and adults, normal, actively infected, and convalescent poliomyelitis monkeys, normal rabbits, and normal and artificially immunized horses, goats, and sheep. Serums were also obtained from rabbits that had been systematically immunized with different bacterial species. A high agglutinating titer was demonstrated in these serums toward the homologous organisms (Table II).

Normal Serums—Adults and Children.—One hundred serums from supposedly normal adults without a history of poliomyelitis infection gave positive tests in 64 instances (64 per cent). The negative group included diphtheria immunes (Schick negatives) and typhoid immunes (Widal strongly positive). One hundred and thirty-six children ranging in age from six weeks to nineteen years gave negative serum reactions in 111 cases (81.6 per cent). In this negative group were in-

cluded 102 serums having demonstrable antibodies for diphtheria (94), typhoid fever (5), and pneumonia (3).

In Table I are summarized the results of tests on children and adults, with reference to degree of reaction and different age groups in children.

Normal Monkey Serums—79.—Negative tests were observed in 66 (83.6 per cent) serums from these animals, and 13 were questionably slightly positive by test for antibodies in varying degrees. These positive serums were not controlled in the usual manner by virus absorption tests and the method of collecting these was not known.*

Normal Horse Serums—8.

Normal Goat Serums—4.

Normal Sheep Serums—2.

Normal Rabbit Serums—12.

In this group of 26 serums, one was strongly positive, four moderately positive, four weakly positive, and five negative. Three negative tests out of eight were obtained with horse serum and both specimens from sheep were likewise negative, as were those from rabbits.

Comment.—It would appear that serum antibodies may be present in certain animals that are naturally refractory or immune to infection with poliomyelitis. Stewart and Haselbauer[2] found normal sheep serum to be sometimes inactivating. They also reported that sheep yielding this serum when subjected to immunization with poliomyelitis virus failed to give a more potent serum. In fact, occasionally the animals lost the power of inactivation originally demonstrated by neutralization tests in the monkey.

There is other experimental evidence of a fundamental nature that has application to these matters under discussion. Bürgi[3] tested the agglutinating power of serums of several species of animals including man, horse, dog, guinea pig, rabbit, goat, sheep, and others against different species of bacteria. It was found that these serums tended to show the same order of potency against these strains of organisms, regardless of which were tested. In further studies he demonstrated the same order of activity when the serums were tested for their property of flocculating suspensions of mastic, in principle analogous to the test with colloidal gold.

Convalescent Serums From Children and Adults—19.—Of these, 6 were strongly positive, 7 moderately strong, 5 weakly positive, and one negative.

In all instances the degree of reaction was seemingly correlated with the clinical course of the disease, as illustrated by some of the following typical clinical histories:

(Children's Hospital, San Francisco, courtesy of Drs. Edward Shaw and Hulda Thelander.)

E. D., aged thirteen years. Symptoms appeared on October 6, 1930, and almost complete paralysis of both legs and right arm developed rapidly. The patient received 50 c.c. convalescent human serum after admission. On December 16, 1930, improvement was marked and the patient was in excellent condition and receiving physiotherapy. The serum, tested on this date, gave the following reaction: 2223345555.

*As this article goes to press, several articles published by C. V. Jungeblut and associates have also shown that neutralizing substances for poliomyelitis virus may be found in *normal adult rhesus monkeys*, while the serum of immature animals reacts uniformly negatively. (Proc. Soc. Exp. Biol. and Med. **29**: 879, 1932.)

R. L., aged four years, developed symptoms and paralysis of left leg on November 10, 1930. Serum was not given. On December 16, 1930, when the patient's serum was tested, the general condition was poor and no improvement had set in. The test was negative: 7777777777.

R. L. M., aged six years, developed symptoms and slight weakness of right leg on November 17, 1930. Fifty cubic centimeters of convalescent serum were given after admission and no progression of weakness developed. On December 16, 1930, the patient was in good condition and was receiving physiotherapy. Serum tested on this date was positive for immune bodies: 2223344555.

S. F., aged seven years, developed symptoms and paralysis of left arm and leg on December 14, 1930. Fifty subic centimeters convalescent serum were given after entry into hospital. Serum for test was drawn on February 6, 1931, when the patient was convalescent, in good condition, and receiving physiotherapy. The serum was strongly positive for immune bodies: 1111143333.

A. C., aged seven years, had been ill for three days prior to admission into the Children's Hospital on February 21, 1931. Injections of 50 c.c. of convalescent serum had been given on the nineteenth and twentieth of February when marked weakness of the legs developed and progressed to all extremities and respiratory muscles. Transfusion of 100 c.c. of convalescent blood and injection of 100 c.c. of sheep serum were given on February 21. The diaphragm and intercostal muscles were completely paralyzed and the child was kept in the Drinker respirator almost constantly. For the past ten weeks the child has been treated in the respirator for eight to ten hours daily. The diaphragm shows virtually no movement and the arms and legs only fair recovery. At this time (May 4, 1931) the serum test was positive: 1112377777.

A. R., aged twelve years, admitted to hospital on March 7, 1931, had been sick eighteen hours with preparalytic symptoms. Lumbar puncture yielded cerebrospinal fluid having 31 cells, 81 per cent lymphocytes. Between March 7 and 10, 165 c.c. convalescent serum and 140 c.c. sheep serum were given. On March 12 generalized paralysis developed, six days after onset of the disease. The intercostal muscles were also involved and the child was treated in the respirator intermittently for three weeks. The response of extremities and respiratory muscles has been very satisfactory. Serum test on May 4, 1931, was positive: 1112357777.

The next two cases in twins illustrate the difficulties often encountered in diagnosis, particularly when the disease occurs in the same household.

P. D., aged eight years, on April 12, 1931, developed fever of 102° to 105°, photophobia, headache, vomiting, and pains in the neck and back. Slight ptosis of one eye and general hyperesthesia completed the picture on April 17, 1931, when the patient was admitted to the hospital. The cerebrospinal fluid contained 150 cells, 69 per cent lymphocytes. Forty-five cubic centimeters sheep serum was given. Four days later, April 21, 1931, the temperature became normal and no weakness developed. The cerebrospinal fluid contained 84 cells with 67 per cent lymphocytes. The recovery was uneventful and a serum test was made on May 4, 1931, two weeks after improvement first set in, and was slightly positive: 3335577777. June 29, eight weeks later, the test was similar: 3337777777.

M. D., aged eight years, twin brother of the preceding patient, developed fever, headache, and pain in the back of the neck one day after the brother became ill, and stayed in bed thirty-six hours. He complained of weakness and had difficulty in walking. During the ensuing nine days until he entered the Children's Hospital on April 22, he limped from time to time when tired. Examination revealed normal reflexes and a slight toe drop on the right foot in association with a "limp." A diagnosis of probable poliomyelitis was made at this time, but no serum treatment was given to patient. A serum test made on May 4, 1931 was negative: 7777777777, but on June 29, eight weeks later, the colloidal gold test was: 1112233444. This definitely positive test with the serum at a time when immune substances have had an opportunity to develop suggests quite strongly that the original diagnosis of poliomyelitis was correct.

(Mount Zion Hospital, San Francisco, courtesy of Dr. Emil Jellinek.)

M. G., aged twenty-four, developed symptoms of weakness and gastrointestinal disturbance with marked weakness of upper extremities. One hundred cubic centimeters pooled convalescent

serum was given after admission in October, 1930. The patient improved rapidly and was convalescent after three weeks. The patient's serum was tested during the first, second, and third weeks of illness and was strongly positive for immune bodies. No paralysis developed.

A. M., aged twenty-eight, entered the hospital in October, 1930, with profound prostration and advanced paralysis of the upper extremities. The cerebrospinal fluid showed a cell count of over 1,600 lymphocytes. Bulbar symptoms developed during the first week and paralysis extended to the accessory respiratory muscles and the lower extremities. The patient's serum was tested during the first week of illness and gave a negative reaction: 7777777777. During the fourth week, there was no change and after two months, the reaction was weakly positive: 3337777777. Convalescence was protracted and after six months there was marked residual paralysis of the arms.

Comment.—The foregoing observations in patients suggest a relationship between the clinical progress of the disease and the antibody content of the serum. The course of the infection, its duration and outcome in these examples were in close agreement with the observed tests made on the different serums obtained from these patients. In all respects the results harmonized with those obtained in experiments on monkeys.

Convalescent and Actively Infected Monkeys—51.—Strongly positive reactions were obtained in 15, moderately strong in 12, weakly positive in 13, and negative tests in 11. Of the last named, a few of the animals showed no clinical improvement whatsoever and died; the remaining were prostrated and in poor condition at the time the serums were tested.

Comment.—Prior to inoculation with poliomyelitis virus, all these animals gave typical negative serum reactions. Clinical improvement and progressively stronger reactions appeared during the paralytic stage and during established convalescence as the prognosis became favorable. In a number of animals, subclinical doses of virus failed to cause infection but the serum test showed antibody content in varying degrees. This is in conformity with the observations made during epidemics of poliomyelitis and is also suggestive of the immunity developed by adults without knowledge of definite exposure.

For purposes of illustration and comparison, a brief clinical description is given of a few of the monkeys in this experiment:

Macacus rhesus No. 3 received intracerebrally on December 5, 1930, 1 c.c. of a 5 per cent virus filtrate obtained from the Hooper Laboratories, University of California. On December 23, 1930, there was partial paralysis of the right arm and leg. On the following day, complete paralysis of the extremities on both sides had occurred. Serum tests were made eight times between December 24, 1930, and January 4, 1931, and gave entirely negative results, reading 7777777777. On January 5, 1931, the animal was moribund and etherized. Autopsy disclosed typical lesions of poliomyelitis confirmed by histologic examination of the brain and cord.

Macacus rhesus No. 4 received intracerebrally on December 22, 1930, 0.75 c.c. of a 10 per cent virus filtrate (Hooper Laboratory strain). A serum test made a few days previously was negative: 7777777777. No symptoms developed until January 28, 1931, about five weeks after inoculation, when the animal appeared somewhat slow in its movement and vomited during the day. There was some excitability and ruffled fur. No other changes were noticed and no paralysis developed. A serum test on February 17, 1931, was moderately positive: 222456777; and at the end of February the animal was still normal in appearance and behavior. Another serum test was again positive.

Macacus rhesus No. 7 was inoculated intracerebrally on February 5, 1931, with 0.5 c.c. of a 5 per cent virus filtrate (Flexner strain). The serum test on this date was negative: 7777777777. Six days later, February 11, 1931, the animal showed slight weakness of right leg with some ataxia. February 16, 1931, on the eleventh day following inoculation, all extremities were completely par-

alyzed. The serum test on February 17, 1931, was positive: 1111223467. During the next two weeks there was steady improvement and the test on February 28, 1931, was strongly positive: 1111112244. One month later the animal had regained the use of arms and legs to some degree and was able to hold food in its hands. The serum was tested again on March 26, 1931, and gave the reading: 1111122333. Toward the end of April, 1931, the animal showed progressive improvement and was able to move about fairly well.

Comment.—These observations were instructive with regard to the seeming relationship between the clinical course of experimental poliomyelitis in the monkey and in the vitro test for antibodies in the serum.

Three distinct clinical features are illustrated in this experiment. One animal represented a rapidly progressing poliomyelitis in which no improvement whatever occurred and was followed by death. A second monkey showed only transitory symptoms of the disease that ordinarily might escape notice. The third animal developed typical poliomyelitis that went on to progressive recovery. These characteristics find a counterpart in the human type of infection and are in all respects similar.

From the standpoint of prognosis and immune bodies in the serum the experimental results appear to be consistent. The same observations had already been made with our serums from convalescent patients and in the development of serum antibodies among "normal" adults.

Although more than one factor may be implicated, it would seem that the serum test for poliomyelitis antibodies confirms certain observations that have suggested mass immunization as one of the factors during epidemics and at other times. As a further corollary it is clear that unrecognized infections or abortive poliomyelitis may give rise to serum antibodies that are detectable by the colloidal gold test in supposedly normal adults.

Immune Monkey Serums—32.—Tests with these serums resulted as follows: 13 were strongly positive, 15 moderately strong, three weakly positive, and one negative.

Immune Horse Serums—8.

Immune Goat Serums—2.

Immune Sheep Serums—2.—The tests with goat serums were moderately or weakly positive and resembled normal goat serum in this respect. The sheep serums yielded slightly positive reactions.

Serums from artificially immunized horses (Parke and Weyer) showed striking and consistently positive reactions. One part (unit) of these serums had a known protective action against 2 to 10 and as high as 25 units of potent poliomye-

SERUM	POTENCY*	COLLOIDAL GOLD TEST
Polio Horse 4283	25 - 1 to 20 - 1	1111123577
Polio Horse 5268	5 - 1 to 20 - 1	1111122557
Polio Horse 4728	10 - 1	1111111122
Polio Horse "Tom"	20 - 1	1115577777
Polio Horse 5102	25 - 1	1111235677
Polio Horse 5259	2 - 1	2222223345
Polio Horse "Concentrate"	100 - 1	1111111111
Polio Sheep No. 117	?	5566677777
Polio Goat No. 3	?	5566667777

*Expressed in terms of number of parts of virus neutralized by one part of serum as determined by intracerebral injections in M. rhesus monkeys.

litis virus. Two samples of "concentrate," effective against 100 doses of virus, also gave prompt and complete precipitation reactions. Controls (ether tricresol, 0.4 per cent, to check effect of the preservative) were negative.

TABLE II

RESULTS OF COLLOIDAL GOLD TESTS WITH DIFFERENT SERUMS

SERUM	NUMBER	POSITIVE	PER CENT	NEGATIVE	PER CENT
Normal:					
Adult	100	64	64.0	36**	36.0
Child	136	25 (e)	18.4	111*	81.6*
Monkey	79	13 (a)	16.4	66	83.6
Horse	8	5 (b)	62.5	3	37.5
Goat	4	4 (c)	100.0	0	0.0
Sheep	2	0	0.0	2	100.0
Rabbit	12	0	0.0	12	100.0
Convalescent:					
Adult	3	3	100.0	0	0.0
Child	16	16	100.0	0	0.0
Monkey	51	39	76.5	12 (d)	23.5
Immune:					
Monkey	32	31	96.9	1	3.1
Horse	8	8	100.0	0	0.0
Goat	2	2	100.0	0	0.0
Sheep	2	2	100.0	0	0.0
		0	0.0	10	100.0

Rabbit Antiserum:		
Staphylococcus	2	
Streptococcus	1	
Polyvalent Staphylococcus and Streptococcus	1	
Typhosus	1	
Paratyphosis A	1	
Paratyphosis B	1	
Enteritidis	1	
Pneumococcus I	1	
Pneumococcus II	1	
	10	
Total	465	

*This group included 94 Schick negatives (diphtheria immunes), 5 typhoid immunes and 3 pneumonia immune serums.

**This group included Schick negatives and Widal positives.

(a) Serums obtained from another institution. These did not show a clean-cut reaction and were not checked for specificity by means of virus absorption test.

(b) Of these, four were only moderately positive.

(c) Of these, one was moderately positive and three weakly positive.

(d) These tests were made during the paralytic stage in monkeys that were not gaining and in those that died shortly afterward.

(e) Weakly positive results in four cases and moderately strong in eight (in age group nine to nineteen years). Strongly positive in eight cases (in age group fourteen to nineteen years).

Immune Rabbit Serums—10.—These serums were obtained from a series of rabbits that had been systematically immunized with one of the following organisms: staphylococcus, streptococcus (one animal received a mixture of polyvalent streptococcus and staphylococcus), pneumococcus, and organisms of the typhoid, paratyphoid, and enteritidis group.

The colloidal gold reaction was entirely negative with this group of serums that represented antibodies for diseases other than poliomyelitis.

The results confirmed the observations also made with human serums that reacted negatively despite a high antibody content for diphtheria or typhoid fever or both.

Comment.—The results obtained with the colloidal gold test on 465 serums, and summarized in Table II, appear to warrant the conclusion that the method described is applicable to the study of poliomyelitis antibodies in serum.

The experimental findings also support certain hypotheses and some definite observations that have been made in the past and related particularly to the neutralizing power of serums from supposedly normal persons.

Our observations in this respect are in conformity with the earlier experiments of Anderson and Frost,[4] and Zingher,[5] and, more recently, Shaughnessy and associates,[6] Aycock and Kramer,[7] and Rhoads.[8] The inactivating power of such serums for poliomyelitis virus may equal and even surpass the neutralizing capacity of serums from persons that have recovered from an attack of the disease. This holds true for certain animal serums also, notably those derived from naturally immune or refractory species.

In the age groups including a number of infants and children up to eight years, the test has failed to demonstrate immune substances in the blood serum, except in rare instances in which weakly positive reactions occurred. This is consistent with our knowledge of susceptibility to this disease during early childhood.

Study of serums from convalescent patients and monkeys brought out facts suggesting a relationship between development of antibodies and prognosis in poliomyelitis.

In order to appreciate the general significance of the data under consideration, it may be well to formulate certain questions.[9] Are the normal antibodies acquired or inherited? If genetic factors are most important, will the normal antibodies appear at a certain stage of development independently of external stimuli, or is the inherited factor merely a capacity for response to a given stimulus by production of antibodies? This implies a response of some kind regardless of whether or not the environmental factor is concerned with exposure to poliomyelitis.

There are data available in other infectious diseases such as diphtheria and in the abundant studies on normal hemagglutinins and hemolysins. It is well known, for instance, that normal agglutinins are found more commonly in the serum of adult men and animals than in the young. The experiences with the Schick reaction in the case of diphtheria antitoxin are decidedly informing with respect to antibody content of serum in certain age groups. Hirszfeld[10] does not regard Zingher's data[11] on the Schick test as necessarily proving that an infection with the diphtheria bacillus was the specific stimulus. We do not know how often such a stimulus is applied in the form of infection producing no clinical symptoms.

It is conceivable that the gradual development of the antibody-producing mechanism varying with age may play a part equally as important as the chances of receiving a specific environmental stimulus. There are facts in favor of this idea. For example, the high resistance to infection with pneumococcus found in the normal fowl is associated with type-specific antibodies in the blood. It would be necessary to assume that in a short space all fowls receive specific stimuli with the different antigenic elements of the pneumococcus. Similarly, it is difficult to explain the resistance of the dog, cat, and sheep and the susceptibility of the mouse, rabbit, and man to pneumococcus infection on the basis that the first named group are infected with the organism frequently and the latter only rarely. These differences

in resistance, however, are associated with variations in antibody content and the ease or difficulty with which artificial immunity may be effected.

CONFIRMATION OF IN VITRO TEST BY IN VIVO NEUTRALIZATION EXPERIMENTS

The nature of the precipitation phenomenon exhibited by certain serums was studied more minutely by means of in vivo neutralization experiments in the monkey. It was especially desirable to evaluate the protective property of serum obtained from normal adults. As is now known such persons constitute a fairly large group having serum with demonstrable neutralizing power.

For this purpose two serums were selected from a group of normal adults, among whom over 60 per cent showed immune bodies in their serum by the in vitro test. One serum giving a negative, and the other a definite positive result with the colloidal gold test were employed.

In the experiments to be recorded, a highly potent virus obtained from the Rockefeller Institute through the courtesy of Dr. Simon Flexner was used. This virus caused typical poliomyelitis within seven days after intracerebral inoculation of 0.5 c.c. or less of a 5 per cent emulsion filtrate prepared from the brain and cord. The strain of virus had been passed through three monkeys with fairly uniform results in our hands.

The virus filtrate was mixed with 0.5 c.c. serum (previously inactivated for one-half hour at 56° C.), incubated at 37° C. for one hour, allowed to stand overnight in the icebox (4° to 6° C.), and the entire contents injected intracerebrally under ether anesthesia and careful surgical technic. Under these conditions, immune serums neutralize the virus whereas strictly normal sera do not.

April 18, 1931, six monkeys were employed; two received normal, unpreserved human serum giving a negative test (7777777777), two others a human serum with a positive test (1111224477) and the remaining two received the virus alone. Table III gives the results. The control animals became paralyzed on the sixth to the eighth day, the animals that received the strictly normal serum came down on the seventh to the ninth day, and the two animals receiving the positive test serum remained free of all symptoms.

Comment.—Under the conditions of the experiment, normal adult human serum inactivated the virus only when the colloidal gold test was positive and indicated the presence of immune bodies. In this instance the serum was effective against approximately 50 to 60 M. L. D., as calculated on the usual activity of the virus.

These experiments suggested the possibility of utilizing the colloidal gold test to evaluate mixtures of serum and virus for purposes of immunization. Such mixtures of serum and virus that failed to induce poliomyelitis infection in monkeys might prove useful in developing an active immunity. One form of a crucial test would depend upon the outcome of a second inoculation in such protected monkeys with a dose of virus capable of bringing down untreated control animals. A second and more satisfactory method would be that of testing the virucidal property of serums from such monkeys. Such studies, now in progress, aim to use a quantity of virus combined with serum in appreciable excess so as to yield a positive precipitation reaction with these "protected" mixtures.

TABLE III

NEUTRALIZATION EXPERIMENTS IN MONKEYS WITH SERUMS TESTED BY COLLOIDAL GOLD METHOD

M. RHESUS NO.	MATERIAL TESTED	AMOUNT c.c.	VIRUS AMOUNT c.c.	RESULTS
9	Unpreserved, normal adult serum giving positive colloidal gold test (1111224477)	0.5	0.5 (10% filtrate)	No symptoms; remained well
10	Unpreserved, normal adult serum giving positive colloidal gold test (1111224477)	0.5	0.62 (20% filtrate)	No symptoms; remained well
11	Unpreserved, normal adult serum giving negative colloidal gold test (7777777777)	0.5	0.5 (10% filtrate)	Typical poliomyelitis, 9 days
12	Unpreserved, normal adult serum giving negative colloidal gold test (7777777777)	0.5	0.62 (20% filtrate)	Typical poliomyelitis, 7 days
13	—	—	0.5 (10% filtrate)	Typical poliomyelitis, 8 days
14	—	—	0.5 (20% filtrate)	Typical poliomyelitis, 6 days; died on seventh day

VIRUS ABSORPTION EXPERIMENTS AND THEIR APPLICATION TO SPECIFIC SERUM POTENCY

Experiments were devised to study the possible application of the colloidal gold test to the degree of neutralization in vitro of poliomyelitis virus by specific immune serums.

Preliminary attempts were made by combining virus filtrate as a serum diluent before addition of the gold sol and electrolyte. The effect was to prevent precipitation and the serums behaved in this respect like normal negative monkey serum.

The method adopted for quantitative study utilized a number of our immune monkey serums and potent antipoliomyelitis horse serums obtained through the kindness of Dr. William H. Park, New York City Department of Health.[12] Five and 10 per cent virus emulsions (Flexner strain) were prepared with doubly distilled water and centrifuged for ten minutes at 3,000 revolutions per minute. Dilutions of virus were made from the supernatant fluid and distributed in a series of test tubes. To these was added next to the least amount of serum found by previous tests to cause complete precipitation of the colloidal gold according to the regular method. After one hour incubation of 37.5° C. the colloidal gold and sodium chloride solution were added and the results read twenty-four hours later. Controls included normal serum, poliomyelitis serum in the same amounts without virus, and dilutions of encephalitis (Levaditi) and herpes virus (Goodpasture) obtained through the kindness of Dr. Schultz of Stanford University.

The effects of adding virus to serums were studied in the following manner: (1) Different concentrations of virus in fixed amounts were combined with varying dilutions of serum. (2) Different dilutions of a 5 per cent or 10 per cent virus in fixed amounts were combined with fixed amounts of serum.

The following protocols illustrate typical experiments and are summarized in Tables IV and V. The double barred line indicates the transition from definite precipitation (complete or partial) to absence of precipitation (slightly positive to completely negative zones).

TABLE IV

VIRUS ABSORPTION TESTS AND EFFECT ON COLLOIDAL GOLD REACTION

SERUM (A) C.C.	VIRUS (A) C.C.	NONE	5%	10%	20%	NORMAL SERUM
0.2	0.05	2	2	3	7	7
0.15	0.05	2	2	7	7	7
0.1	0.05	2	2	7	7	7
0.1 (1-2)	0.05	2	7	7	7	7
0.1 (1-4)	0.05	2	7	7	7	7
0.1 (1-6)	0.05	2	7	7	7	7
0.1 (1-8)	0.05	2	7	7	7	7
0.1 (1-10)	0.05	2	7	7	7	7
0.1 (1-20)	0.05	2	7	7	7	7
0.1 (1-32)	0.05	3	7	7	7	7

(a) Serum from convalescent M. rhesus No. 7 and poliomyelitis virus (Flexner strain).

TABLE V

EFFECT OF VIRUS ABSORPTION ON COLLOIDAL GOLD REACTION, USING POLIOMYELITIS VIRUS AND OTHER VIRUSES COMBINED WITH POLIOMYELITIS IMMUNE AND NORMAL SERUM

SERUM (A) C.C.	5% VIRUS (A) C.C.	NONE		AFTER ABSORPTION WITH VIRUS					
				POLIOMYELITIS		HERPES		ENCEPHALITIS	
		POLIO	NORMAL	POLIO	NORMAL	POLIO	NORMAL	POLIO	NORMAL
0.2	0.05	2	7	2	7	2	7	2	7
0.15	0.05	2	7	2	7	2	7	2	7
0.1	0.05	2	7	2	7	2	7	2	7
0.1 (1-2)	0.05	2	7	7	7	2	7	2	7
0.1 (1-4)	0.05	2	7	7	7	2	7	2	7
0.1 (1-6)	0.05	2	7	7	7	2	7	3	7
0.1 (1-8)	0.05	2	7	7	7	3	7	3	7
0.1 (1-10)	0.05	2	7	7	7	3	7	3	7
0.1 (1-20)	0.05	2	7	7	7	3	7	3	7
0.1 (1-32)	0.05	2	7	7	7	3	7	3	7

(a) Serum from convalescent M. rhesus No. 7, poliomyelitis virus (Flexner strain), and herpes (Goodpasture strain) and encephalitis virus (Levaditi strain).

In the following experiments illustrated by specimen protocols in Tables VI and VII virus absorption tests were applied to serums of different potencies. The technic differed somewhat from the preceding series of tests in that the cholesterinized sodium chloride solution (0.4 per cent) was added along with the colloidal gold to the previously incubated mixtures of serums and different viruses. This

method was found better suited to sharper end-points in the reading of the absorption tests, and this procedure was adopted.

Comment.—These observations indicate that the precipitation of colloidal gold by immune serums does not occur in the presence of poliomyelitis virus. The phenomenon is apparently dependent upon the amount of virus used for absorption and the antibody content of the serums in question. By varying the concentration of one or the other, singly, the reversal of reaction can be demonstrated (Tables IV, V, and Va).

TABLE Va

EFFECT OF VIRUS ABSORPTION ON COLLOIDAL GOLD REACTION, USING POLIOMYELITIS VIRUS COMBINED WITH VARYING AMOUNTS OF CONVALESCENT POLIOMYELITIS SERUM

(A) SERUM C.C.	VIRUS (10%) C.C.	REACTION (B)	
		BEFORE ABSORPTION	AFTER ABSORPTION
0.05	0.2	1	5
0.05	0.15	1	1
0.05	0.1	1	1
0.05	0.1 (1-2) (0.05)	1	1
0.05	0.1 (1-4) (0.025)	1	1
0.05	0.1 (1-6) (0.016)	1	1
0.05	0.1 (1-8) (0.012)	1	1
0.05	0.1 (1-10) (0.01)	1	1
0.05	0.1 (1-20) (0.005)	1	1
0.05	0.1 (1-32) (0.003)	1	1
0.03	0.2	1	7
0.03	0.15	1	3
0.03	0.1	1	3
0.03	0.1 (1-2) (0.05)	1	2
0.03	0.1 (1-4) (0.025)	1	2
0.03	0.1 (1-6) (0.016)	1	2
0.03	0.1 (1-8) (0.012)	1	1
0.03	0.1 (1-10) (0.01)	1	1
0.03	0.1 (1-20) (0.005)	1	1
0.03	0.1 (1-32) (0.003)	1	1
0.02	0.2	1	7
0.02	0.15	1	7
0.02	0.1	1	7
0.02	0.1 (1-2) (0.05)	1	3
0.02	0.1 (1-4) (0.025)	1	3
0.02	0.1 (1-6) (0.016)	1	3
0.02	0.1 (1-8) (0.012)	1	3
0.02	0.1 (1-10) (0.01)	1	2
0.02	0.1 (1-20) (0.005)	1	1
0.02	0.1 (1-32) (0.003)	1	1

(a) The serum from an adult female patient that had recovered fully from a severe attack of poliomyelitis, was highly potent and small amounts neutralized 0.5 c.c. of a 5 to 10 per cent filtrate of an active Flexner strain of virus.

(b) Note the increased precipitation effect as the amount of virus is diminished, and the wider range of more complete precipitation in the presence of greater excess of antibodies. In this and in all other tables the colloidal gold reaction is indicated by numbers as described in the text.

The underlying mechanism of this phenomenon and the principle involved lend themselves to experimental study in various ways. It seems likely that when the antibody is in excess due to incomplete absorption, the serum will still give a

TABLE VI

VIRUS ABSORPTION TESTS AND COLLOIDAL GOLD REACTION WITH VARIOUS ANTIPOLIOMYELITIC HORSE SERUMS

A AFTER VIRUS ABSORPTION		B BEFORE VIRUS ABSORPTION									
		POLIOMYELITIS IMMUNE SERUMS									
SERUM (A) C.C.	VIRUS (10%) (A) C.C.	4283		4728		"TOM"		5102		5259	
		A	B	A	B	A	B	A	B	A	B
0.05	0.00	1	1	1	1	1	1	2	1	2	2
0.05	0.15	4	1	3	1	5	1	5	1	5	2
0.05	0.1	4	1	3	1	5	1	5	1	4	2
0.05	0.1 (1-2)	3	1	2	1	5	5	4	1	3	2
0.05	0.1 (1-4)	2	1	1	1	1	5	3	2	2	2
0.05	0.1 (1-6)	1	2	1	1	1	7	3	3	2	2
0.05	0.1 (1-8)	1	3	1	1	1	7	3	5	2	3
0.05	0.1 (1-10)	1	5	1	1	1	7	3	6	2	3
0.05	0.1 (1-20)	1	7	1	2	1	7	2	7	2	4
0.05	0.1 (1-32)	1	7	1	2	1	7	2	7	2	5

(a) Serum from poliomyelitis horses (Park and Weyer) and poliomyelitis virus (Flexner strain).

TABLE VII

VIRUS ABSORPTION TESTS WITH DIFFERENT VIRUSES AND POLIOMYELITIS IMMUNE SERUMS

CONVALESCENT SERUM (20-1)			10% VIRUS (A) C.C.	SERUM (A) C.C.	HORSE SERUM (2-1)		
POLIOMYELITIS	ENCEPHALITIS	HERPES			POLIO.	ENCEPH.	HERPES
3	3	3	0.2	0.05	7	2	3
3	3	3	0.15	0.05	7	2	3
2	3	3	0.1	0.05	7	2	2
2	3	3	0.1 (1-2)	0.05	6	2	2
2	3	3	0.1 (1-4)	0.05	3	2	2
2	3	3	0.1 (1-6)	0.05	3	2	3
2	3	3	0.1 (1-8)	0.05	2	–	2
2	3	3	0.1 (1-10)	0.05	2	–	2
2	3	3	0.1 (1-20)	0.05	2	–	2
2	3	3	0.0	0.05	2	–	2

(a) Human convalescent serum; horse serum (immune 5259); poliomyelitis virus (Flexner); herpes (Goodpasture) and encephalitis viruses (Levaditi).

positive precipitation test. The factors that modify the outcome of this absorption are doubtless the amount of virus on the one hand and, on the other, the potency of the serum. If this be correct, the effective neutralizing power of a given serum as demonstrated by this method ought to be shown by the result of intracerebral injections of suitable mixtures into M. rhesus monkeys.

Preliminary experiments so devised have already borne out this hypothesis. For example, serum-virus mixtures corresponding to the contents of tubes showing definite precipitation (reading "1") and those giving definitely negative tests (reading "7") in an absorption series were inoculated into monkeys. The results, although purely tentative, indicate the presence of adequate protective antibodies

in serum-virus mixtures that give a positive precipitation test, whereas typical poliomyelitis results from injection of mixtures yielding negative tests in vitro.

The procedure was essentially as follows: Mixtures of given serums, serially diluted, and 10 per cent virus suspensions were subjected to the colloidal gold test. Combinations were selected according to the presence or absence of positive precipitation indicative of "protected" or "unprotected" mixtures, respectively. The material employed for intracerebral injection into macacus rhesus monkeys was uniformly made up to a volume of 0.8 c.c. Suitable controls included mixtures of strictly normal serum and virus and additional virus controls combined with physiologic salt solution only. The strain of active virus used had been preserved in 50 per cent glycerol for seven months.

The results are summarized in Table VIII.

Pertinent to this discussion is the possible effect of adsorbing bodies on the inactivating substances in serum. Rhoads[13] has already shown that suspensions of normal monkey brain are entirely devoid of the power to remove the inactivating

TABLE VIII

NEUTRALIZATION EXPERIMENTS IN MONKEYS WITH "PROTECTED" MIXTURES OF SERUM AND VIRUS. TESTED BY COLLOIDAL GOLD METHOD

M. RHESUS NO.	MATERIAL TESTED	AMOUNT C.C.	VIRUS AMOUNT C.C.	RESULTS
5	Convalescent adult serum giving positive colloidal gold test ("protected" mixture)	0.46	0.2	No symptoms; remained well
15	Physiologic salt solution	0.6	0.2	Typical poliomyelitis, sixth day; died on seventh day
16	Convalescent adult serum giving positive colloidal gold test ("protected" mixture)	0.3	0.2	No symptoms; remained well
21	Convalescent adult serum giving negative colloidal gold test ("unprotected" mixture)	0.02	0.2	Typical poliomyelitis, twelfth day; complete paralysis
22	Convalescent adult serum giving negative colloidal gold test ("unprotected" mixture)	0.04	0.2	Typical poliomyelitis; prostrate eighth day
24	Convalescent adult serum giving positive colloidal gold test ("protected" mixture)	0.6	0.2	No symptoms; remained well
25	Physiologic salt solution	0.6	0.2	Typical poliomyelitis, tenth day; prostrate eleventh day
26	Normal adult serum giving negative colloidal gold test (no ppt.)	0.6	0.2	Typical poliomyelitis, eleventh day; prostrate thirteenth day

substances contained in antipoliomyelitis horse serum. In our experiments this possibility was also anticipated and controlled by means of absorption tests with encephalitis and herpes virus filtrate and normal brain filtrates and centrifugalized emulsions. The absorption effects appeared to be specific for poliomyelitis virus only.

Further studies are suggested here in relation to the phenomena of dissociation of virus in serum-virus mixtures, the possible effects of quantitative alterations in globulins and serum proteins, and other problems bearing upon physiochemical factors. The possibility is also suggested of using the colloidal gold test in the selection of "protected" mixtures of poliomyelitis immune serum combined with virus for purposes of active immunization.

DISCUSSION

We do not wish to be misunderstood as assuming that the colloidal gold test described here is absolutely final. The studies are by no means completed and will bear repetition over a period of time. As far as the results have gone they appear to be convincing. To the extent that in an experimental study of this type a certain method of approach must be followed, the results are self-explanatory. The materials selected with this end in view have yielded data that are not inconsistent.

The use of convalescent serum for the treatment of poliomyelitis has led to search for suitable serums from other sources. A large percentage of supposedly normal adults has been found satisfactory in this regard. It is interesting that very young children and infants do not show such neutralizing power of their serum. This is in accord with the facts of greater susceptibility to the disease and is in keeping with the general principles of immunity to infections. A striking analogy is found in diphtheria, for which the Schick test has shown decreasing susceptibility in the higher age groups beyond that of six years.

Attention should be called to the results noted in a group of thirteen out of sixteen normal monkey serums obtained from another institution.* The observations must stand, but an explanation for this discrepancy must wait upon additional demonstrations of the virus-neutralizing power of such serums tested in the monkey as well as upon the technic used in collecting and storing these serums. The colloidal gold test is supposedly an *indicator* of antibodies in the serum without reference to the absolute neutralizing power of serums so tested. There are often experimental discrepancies between in vivo and in vitro inactivating power. The inference is that there may be quantitative variations in immune capacity. Also a human being or animal may yield a neutralizing serum without being adequately protected against a potent strain of virus.

Where antibodies have been demonstrated in the serum of supposedly normal adults or irregularly in animals such as the monkey, the burden of proof must rest on data showing that such "normal" serum is actually protective in the presence of virus. This does not apply in the same degree to refractory animals such as the horse, goat, or sheep in which the disease cannot be reproduced.

We believe that our results with an in vitro method of demonstrating serum antibodies for poliomyelitis are in reasonably good agreement with the clinical and experimental material studied.

*See footnote, p. 172.

The quantitative relationships of serum potency to effective neutralization of virus by animal test have been corroborated by virus absorption technic in vitro with analogous results.

SUMMARY AND CONCLUSIONS

The in vitro colloidal gold test for serum antibodies in poliomyelitis has been applied to 465 different serums from human and animal sources. The material used represented all stages of the infection as well as normal and convalescent individuals.

Experiments have demonstrated the value of the method by comparing the serum tests with the results of in vivo neutralization tests with the same serums in M. rhesus monkeys.

The specificity of the test and its quantitative aspects have been controlled by means of detailed absorption experiments employing poliomyelitis virus with encephalitis and herpes viruses and further comparison with results obtained with human and animal serums having antibodies for diseases other than poliomyelitis.

More than 60 per cent of 100 normal adults and only 18.4 per cent of the 136 normal children tested showed immune substances in the serum. In the last named group, only 8.3 per cent of the serums in the age group of one year or under and eight years were positive; 5 per cent weak; 3.3 per cent moderately strong; and 26.6 per cent of the children between the ages of nine and nineteen years gave positive reactions, of which 10.6 per cent were moderately strong and 10.6 per cent strongly positive. In the adult group (ages twenty-five to fifty years), 30 per cent gave strongly positive and 20 per cent moderately strong reactions. Among 111 negatively reacting sera obtained from children were included 94 diphtheria immunes, 5 typhoid immunes, and 3 pneumonia immunes. The group of 36 negatively reacting adult serums likewise included Schick negatives and strongly positive Widal tests. Antibodies for diseases other than poliomyelitis did not give false positive reactions. This was shown further in tests with serums from immunized rabbits (Tables I and II).

A colloidal gold test has been devised for the detection of immune substances against poliomyelitis in the blood serum. The application of the method in the study of poliomyelitis has been shown to have a direct bearing and practical value in the following problems:

1. Study of susceptibility to this disease among the general population.
2. Selection of donors' serum for therapeutic use in poliomyelitis, especially during outbreaks.
3. Evaluation of therapeutic potency of serums from human and animal sources.
4. Prognosis during the course of poliomyelitis as related to the progressive development or complete absence of serum antibodies.
5. In vitro selection of "protected" mixtures of poliomyelitis immune serum combined with virus for purposes of active immunization.

REFERENCES

1. Eberson, F.: Colloidal Gold Test for Determination and Titration of Immune Bodies in Poliomyelitis, Proc. Soc. Exper. Biol. & Med. **28**: 405, 1931.
2. Stewart, F., and Haselbauer, P.: Virus Neutralization Experiments With Rosenow's and Pettit's Antipoliomyelitic Sera, J. Exper. Med. **48**: 449, 1928.

3. Burgi, E.: Über Bakterienagglutination durch normale Sera, Arch. f. Hyg. **62**: 239, 1907.
4. Anderson, J. F., and Frost, W. H.: Abortive Cases of Poliomyelitis, J. A. M. A. **56**: 663, 1911.
5. Zingher, A.: The Diagnosis and Serum Treatment of Anterior Poliomyelitis, J. A. M. A. **68**: 817, 1917.
6. Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B.: Neutralization of the Virus of Poliomyelitis by Human Sera, Proc. Soc. Exper. Biol. & Med. **27**: 742, 1930.
7. Aycock, W. L., and Kramer, S. D.: Immunity to Poliomyelitis in Normal Individuals in Urban and Rural Communities as Indicated by the Neutralization Test, J. Prev. Med. **4**: 189, 1930.
   Immunity to Poliomyelitis in a Southern Population as Shown by the Neutralization Test, Ibid. **4**: 201, 1930.
8. Rhoads, C. P.: Reinforcement of Convalescent Antipoliomyelitic Serum in the Monkey, J. Exper. Med. **53**: 139, 1931.
9. Topley, W. W. C., and Wilson, G. S.: The Rôle of Humoral Antibodies in Immunity, Principles of Bacteriology and Immunology **2**: Chap. 46, 1929, Wm. Wood & Co.
10. Hirszfeld, L.: Über die Konstitutionsserologie im Zusammenhang mit der Blutgruppenforschung, Ergeb. d. Hyg. **8**: 367, 1926.
11. Zingher, A.: The Schick Test Performed on More Than 150,000 Children in Public and Parochial Schools in New York (Manhattan and the Bronx), Am. J. Dis. Child. **22**: 392, 1923.
12. Weyer, E. R., Park, W. H., and Banzhaf, E. J.: A Potent Antipoliomyelitic Horse Serum Concentrate and Its Experimental Use in Infected Monkeys, J. Exper. Med. **53**: 553, 1931.
13. Rhoads, C. P.: Experimental Study of a Horse Antipoliomyelitis Serum, J. Exper. Med. **53**: 129, 1931.

SEPTICEMIA DUE TO HIGHER BACTERIA*

Case Report and Bacteriologic Findings

Sara Alicia Scudder, B.A., New York, N. Y.

A GROUP of higher bacteria was reported by Scudder and Belding[1] (1929) from cases of urethritis and cervicitis, associated with other organisms in cases of chronic infection purported to be of gonorrheal origin. The organisms have since been found in many instances associated with others in the genitourinary and respiratory tracts (Case reports from the Bellevue-Yorkville Health Demonstration (1930)[2] and from the City Hospital, Welfare Island, N. Y.). Concrete examples may be cited: labial ulcer in a child with Vincent's organisms; tuberculosis of the parotid; E. friedlander pneumonia. In the last instance Encapsulatus friedlander invaded the blood stream and carried in its wake a mixed flora including higher bacteria and N. catarrhalis. Comparative experiments were carried out with similar strains from the respiratory tract and with the streptococcus-pneumococcus types for purpose of identification. Nomenclature for this type of organism has not been established, although it shows consistent morphologic similarity to strepthriceae. Cumulative evidence, however, points to generic relationship to mycobacteriaceae.[3]

Proof of pathogenicity of this type of higher bacterium has been sought since 1927. The present case of rheumatic heart disease which was studied during the summer of 1931 showed no other type of organism in blood culture. Evidence of focal infection in other tissues which might have prepared the way for blood stream invasion could not be proved.

CASE HISTORY

The patient, a white male, was admitted to City Hospital, service of Dr. E. P. Shelby, on June 12, 1931 with a classical history of rheumatic fever. Tonsillectomy had been performed at six years for hypertrophy. In his eighth summer he had "growing pains" and at sixteen to nineteen years repeated attacks of sore throat. At this time cardiac disease was discovered during physical examination for insurance. At twenty-two and twenty-five years respectively there occurred repeated attacks of rheumatic fever with joint involvement. The last illness began at the age of twenty-six and progressed with moderate severity for one year, then very rapidly for the last three months, presenting symptoms of embolic processes. Autopsy was not obtained.

BACTERIOLOGY

A series of blood cultures, June 16, 1931, to July 13, 1931, showed higher bacteria: flocculent growth in broth, radiate colonies in semisolid agar, discrete mold-like and pea-sized colonies in the original blood culture. The colony in blood agar was of the viridans type, although mildly hemolytic in first culture. The number of organisms were about two per cubic centimeter in the first culture and about

*From the Pathologic Laboratory, City Hospital, Department of Hospitals.
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twenty per cubic centimeter in the final culture. Transfer from discrete colonies to small tubes of broth gave radiate or tuft-like colonies subtended by long trailing filaments which reached to the bottom of the tube. Settling of this orientation of growth took place upon slight agitation (Fig. 1).

The following biochemical tests do not aid in identification of the organism: bile solubility, aesculin fermentation or methyl red test. The simplest means of identification are to be found in their radiate colony formation in broth or semi-solid agar, and the study of hanging drop with oil immersion lens for observation

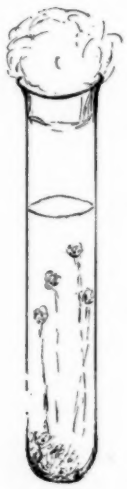

Fig. 1.—Culture in eighteen hours showing long filaments prior to sedimentation.

of filamentous morphology. Serologic differentiation may be shown of value. Detailed report of biochemical and serologic characteristics will be published later in a study of streptococci isolated from cases of rheumatic fever.

SUMMARY

An instance is reported of isolation of a higher bacterium from the blood stream in a case of chronic rheumatic heart disease. The organism is most likely a secondary invader and the direct cause of the terminal clinical features.

REFERENCES

1. Scudder, S. A., and Belding, D. L.: Group of Higher Bacteria From Genitourinary Tract; Preliminary Communication, J. Lab. & Clin. Med. **14**: 801, 1929.
2. Scudder, S. A.: Report to the Medical Committee of the Bellevue-Yorkville Demonstration (Now in press).
3. Bergy's: Manual Determinative Bacteriology, 1923, Williams and Wilkins, p. 388.

LABORATORY METHODS

THE CARE, BREEDING, AND WEIGHT CHANGES OF RABBITS IN THE LABORATORY*

EARL L. BURKY, M.D., BALTIMORE, MD.

FOR the past two years the author has been engaged in the breeding of rabbits under laboratory conditions. The purpose of this breeding has been to establish a strain of rabbits, the heredity of which would be well known before attempting any experiments upon the transmission of acquired characteristics. During this time numerous visitors have commented upon the possibility of raising rabbits in rather limited space and under indoor conditions. As a result of these comments, it seemed worth while to collect the information for publication. It is the purpose of this report, therefore, to summarize the information that has been gathered on the care, breeding, and natural history of rabbits under laboratory conditions.[1]

Breeding Space and Cages.—The room occupied by the animals being bred is 14 by 13 feet. The walls are brick and the floor is waterproofed cement with a drain connection. Two large windows furnish light and ventilation. Fig. 1, roughly drawn to a scale of ¼ inch to the foot, shows the arrangement of the cages.

The animals too young to breed and excess stock are kept outdoors on a ledge surrounding the breeding floor, in two pens, each of which is 12 by 4 feet. The males are kept in one pen and the females in the other. These pens have tile floors with drains. In each pen there is a waterproofed box large enough to hold the food containers. Other than this the animals have no shelter.

The cages in the breeding room are of two types and sizes. The smaller cages are used for housing the males, one animal to a cage. They are 20 by 19½ by 15 inches and made of galvanized iron and wire. Attached to one side of the cage is a hay rack and in the bottom is a removable tray. Food (oats) is kept in galvanized iron cups of the reservoir type, attached to the door by a bolt and nut. The water cup is attached so that the water is entirely outside of the cage. On the outside of the door there is a cardholder in which can be inserted a 3 by 5 inch card (see Fig. 2).

The large cages are used for housing the females and their young. They are 36 by 32 by 18 inches. They differ from the smaller cages in not having a removable pan and the water supply is contained in a poultry-type reservoir.

*From the Wilmer Institute of Ophthalmology, The Johns Hopkins University.
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[1]This work was begun after a visit to the laboratories of Dr. M. F. Guyer at the University of Wisconsin and some of the information herein reported was obtained from Dr. Guyer and his associates.

In these cages, to prevent the spilling of bedding, the front edge of the floor has been flanged to a height of one inch (see Fig. 3).

General Care of Cages.—The cages are cleaned by scraping and brushing the floor twice a week. At least once every two weeks, the entire cage is dropped into a vat of boiling water for about five minutes. The floor of the room is swept

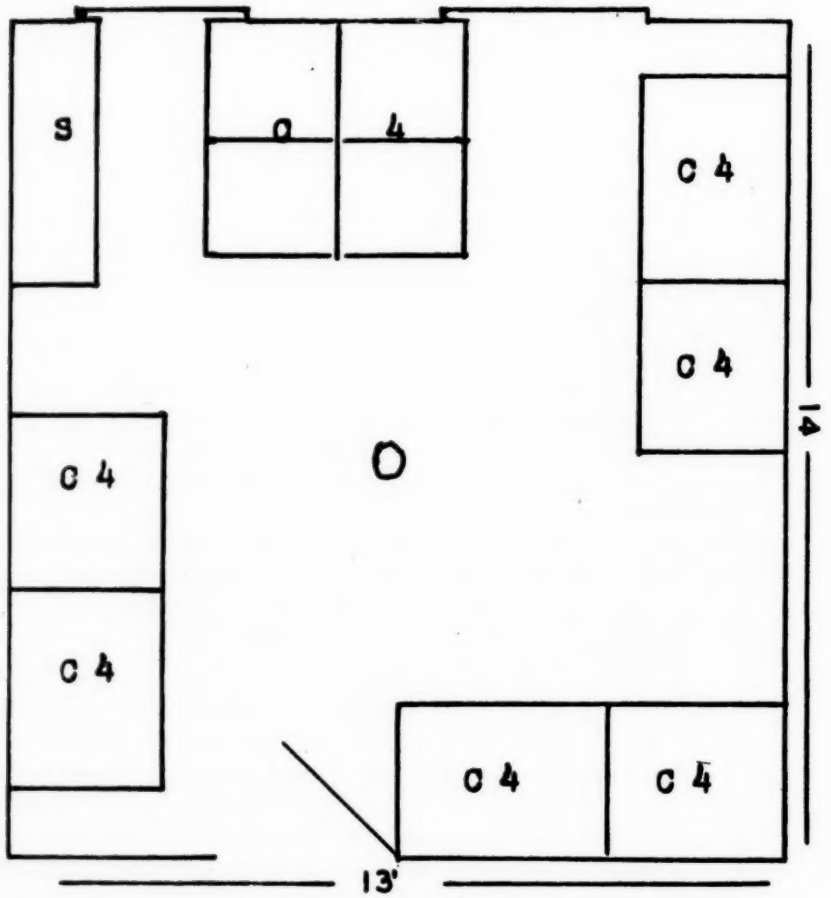

Fig. 1.

at least once daily and is washed with water and a commercial disinfectant at least twice a week. The outdoor pens are washed daily with water during warm weather and as often as the weather permits in cold weather. After each cleaning the floors of the indoor cages are well covered with fresh shavings.

With a cleaning program such as the above no roaches or bedbugs are to be found anywhere on the floor occupied by the animals. During the time this régime has been in operation there have been sporadic and mild cases of snuffles

but no epidemics. Such deaths as have occurred have been mainly in the outdoor pens following heavy rainfalls or periods of intense cold.

Food.—Each cage contains a hay rack which is filled daily with clover and alfalfa hay. Each cage is furnished with a cup of oats daily. Mother and young are fed rolled oats until they are separated. Each adult receives daily a handful of greens in the form of lettuce or cabbage leaves, or beet tops discarded from the hospital kitchens. Animals being weaned are given greens sparingly and the supply is placed in the cage where the greater portion is inaccessible to the young. It has not been necessary to supplement the mother's rations at term to prevent her from killing her young.

Breeding.—The rabbit reaches sexual maturity about the sixth month. At this time the mature animals are moved from the large pens to the breeding cages. At least one week is allowed for the animal to adjust itself to its new surround-

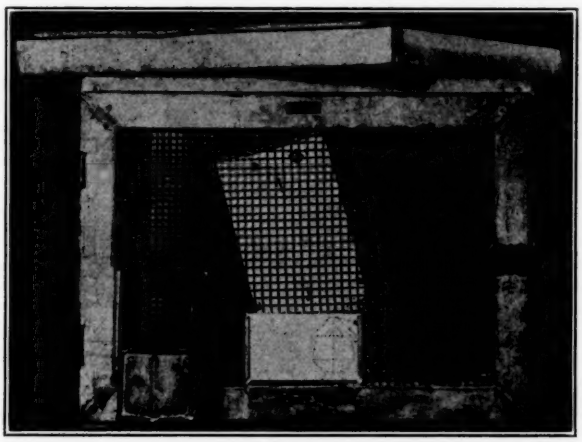

Fig. 2.

ings. The female is then placed in the male's cage. They are watched for a few minutes and if they do not fight they are kept together for twenty-four hours. The female is then returned to the large cage. It is important that this order of events be followed for several reasons. First, if the male is placed in the female's cage he may show more interest in his new surroundings than in the female and in many instances pregnancy does not result if the male is removed in twenty-four hours. Second, if the male shows the proper amatory spirit the female may be disinterested and the larger cage gives her more space to avoid the male.

At the end of the third week the small breeding box is placed in the female's cage. (See Figs. 3 and 4.) This box is made of wood and has one side that turns down so that the interior of the box can be seen without disturbing the young. The mother's entrance is purposely made small so that when she leaves the nest any nursing young clinging to her will be torn loose and not dragged out into the cage where they usually die from neglect.

Sometime during the latter part of the fourth week, the female carries hay into the breeding box and begins pulling fur to make a nest. On the thirtieth or thirty-first day after removal from the male's cage the litter is born. The young

are perfectly helpless until the tenth day when the eyes begin to open. During this period it is inadvisable to handle the young except with forceps and then only to remove dead ones if the mother does not do this. Handling of the newborn with naked hands usually results in the mother either killing or neglecting her litter. If the above routine is followed the mother will seldom kill her young, and then usually only the first litter which in any case is small, four to six, as compared with eight to ten in the later pregnancies.

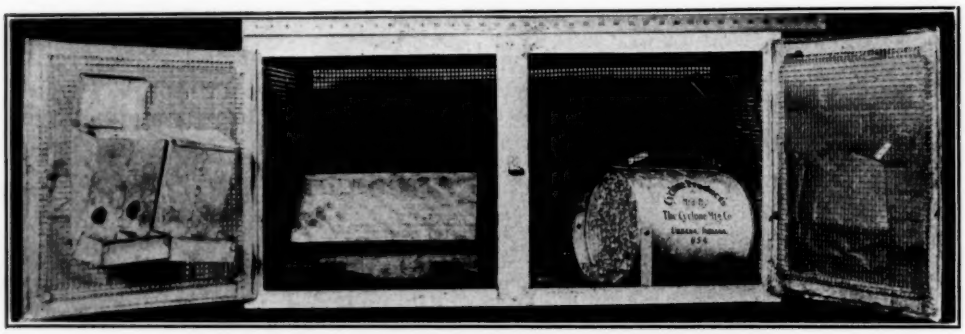

Fig. 3.

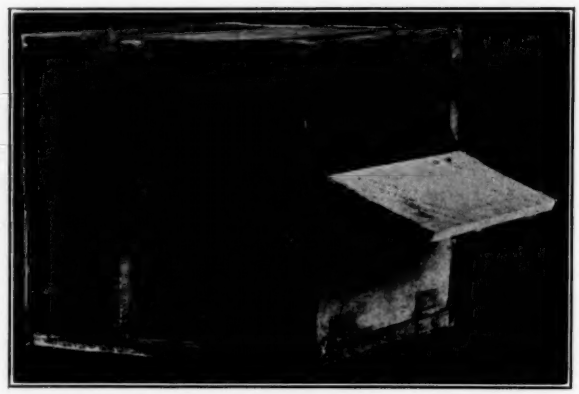

Fig. 4.

On several occasions litters, aged two to five days, have been handled with no ensuing deaths in the following manner. The nest, cage, and mother were sprinkled with strong smelling talcum powder. On the following day the young were handled with rubber gloves that had been sprinkled with the same powder and sterilized in the autoclave. Using this method it has been possible to make subcutaneous injections of various substances which in themselves are not lethal.

A rabbit can be remated within the first week after parturition. If this is done the litter should be weaned about the third week. Such early weaning, however, retards the weight gain and such animals are slow in reaching weight maturity. If several animals litter at the same time, and if for any reason early remating is desirable in one of the group, the young can be moved singly or in pairs daily to the foster-mother's nest, using forceps to handle the young. This

method allows for a slow decrease in the mother's milk supply and places a gradually increased burden on the foster-mother.

Females that are mated frequently are better breeders than those that are mated infrequently and that have gotten overweight. The female rabbit is generally considered to be perpetually in heat. Under cage conditions in Baltimore, however, there is a seasonal variation. From June to October a litter is a rare occurrence. If any young are born, the number is less than usual and they rarely attain maturity. In foretelling successful matings, a dusky, red, swollen appearance of the vaginal mucosa usually indicates a ready acceptance of the buck, but this is by no means a sure sign of a litter. If for any reason it is desirable to experiment on rabbits during pregnancy it is best to use animals that have littered and brought their young to the weaning period successfully on two previous occasions.

The young rabbits are usually kept with the mother for six weeks. It is difficult to differentiate the sexes before the end of the sixth week, but at this time, they are completely weaned and the sexes readily differentiated. They are identified by sex, color markings, and any gross deviations from the normal and are then tagged.

The tags are aluminum bands ordinarily used for pigeon legs. The proper number is stamped on the tag and the tag is inserted into a slit cut by a knife-edge punch parallel with the long axis of the ear and medial to the marginal ear vein. If the cut is made about 1 cm. from the ear margin, and the tag properly closed, the animal rarely loses the marker. The males are tagged in the left ear; the females in the right.

The breeding of large numbers of rabbits and the keeping of proper records demands as simple a system of record keeping as possible, but which is still complete enough to be worth while. In this study we have attempted to keep a record of the ancestry, coat markings, gross deviations from normal, conditions of the eyes, and weight changes, particularly those associated with any pathologic states.

The method of numbering is one commonly used by geneticists while the record cards have been devised by me. All animals purchased from a dealer are given a serial number. When two such animals are mated, the resulting litter is given a symbol. An example is given.

Female 21 × Male 5 = U5A	U = untreated, referring to the experimental procedures on the parents
Individuals in this litter become U5A. 1	5 = the fifth pair of animals mated
U5A. 2	A = the first time this particular pair of animals have been mated
U5A. 3 and so on	
Female U5A. 4 × Male U5A. 1 = U33B	U = as above
	33 = the thirty-third pair of animals mated
	B = the second time this pair of animals has been mated

To record the matings, Form I is used. To record the litters, Form II is used. To record the weights, markings, and experimental procedures, Forms III and IV are used. They are 3 by 5 inches in size and the first three are printed on bond paper. Form IV for use as a marker on the cages is printed on a fairly heavy grade of cardboard. All of these forms are self explanatory and typical records are shown.

Results.—A pair of rabbits was mated in December, 1929. The first litter was born in January, 1930. The brothers and sisters in this litter were first interbred in September, 1930. Mating brother and sister almost exclusively, fifty-four matings were done. Thirty litters were actually born. Of these four litters were destroyed by the mothers. The others were successfully weaned. These litters contained one hundred and twenty-one individuals. Of these, eighty-three are now living and at least three months old. Twelve died as a result of experimental procedures. Ten died following exposure to a severe storm. Sixteen died at varying intervals before attaining an age of six months, from unknown causes. Autopsies performed on the majority of the last group showed nothing but emaciation.

Weight Changes in Rabbits.—While it is rather a common laboratory custom to record the weight changes of experimental animals, there have been few reports published dealing specifically with this subject. Pearce and Van Allen[1] have reported on the gross and organ weight changes in rabbits exposed to various degrees of lighting. Their results suggest that the effect of various environments is reflected in the animal's weight.

Some years ago, as a guide to the injection of proper amounts of bacteria and their toxins for the production of immune substances the weights of the injected rabbits were recorded. It soon became obvious that animals receiving doses larger than the ideal began to lose weight and the immune body production fell off in proportion to the weight loss. Since that time it has been our custom to record the weights of all stock and experimental rabbits twice a week and, if possible, to use no animals that have not been weighed at least twice. Without detailing all of the data that have been gathered it is possible to make a few generalizations.

1. Animals acquired from dealers show a rapid weight increase (200-500 gm.) in their new environment. If such a gain does not occur, it is unwise to begin any lengthy experiments until the animal does show a gain. In most instances when the animal does not show a weight increase within seven to ten days it dies within two to three weeks from either obvious or unknown causes.

2. Under the minor variations of cage environment, the healthy rabbit rarely loses more than 200 gm. in weight.

In Fig. 5 the weight changes of a pair of normal and a pair of experimental animals are recorded. U5A. 1 and U5A. 4 are normal brother and sister and the records shown are similar and almost identical with the records of the other four in this litter. It will be seen that the growth curve for the first year is practically a straight line. It is interesting that slight weight losses occurred when the animals were mated. The degree of weight loss is comparable to that occurring when rabbits are starved for the eighteen hours preceding a bleeding. The most obvious suggestion is that during the mating period there is no time for food.

Following each successful mating there is a significant weight gain of 350 to 500 gm. in the female. This rise is apparent within two weeks after the mating and is the best indicator of the pregnancy. In passing, it may be mentioned that the serum of a two weeks pregnant animal is very turbid and fatty even after a preliminary starvation. These signs indicate a pregnancy, but the young may

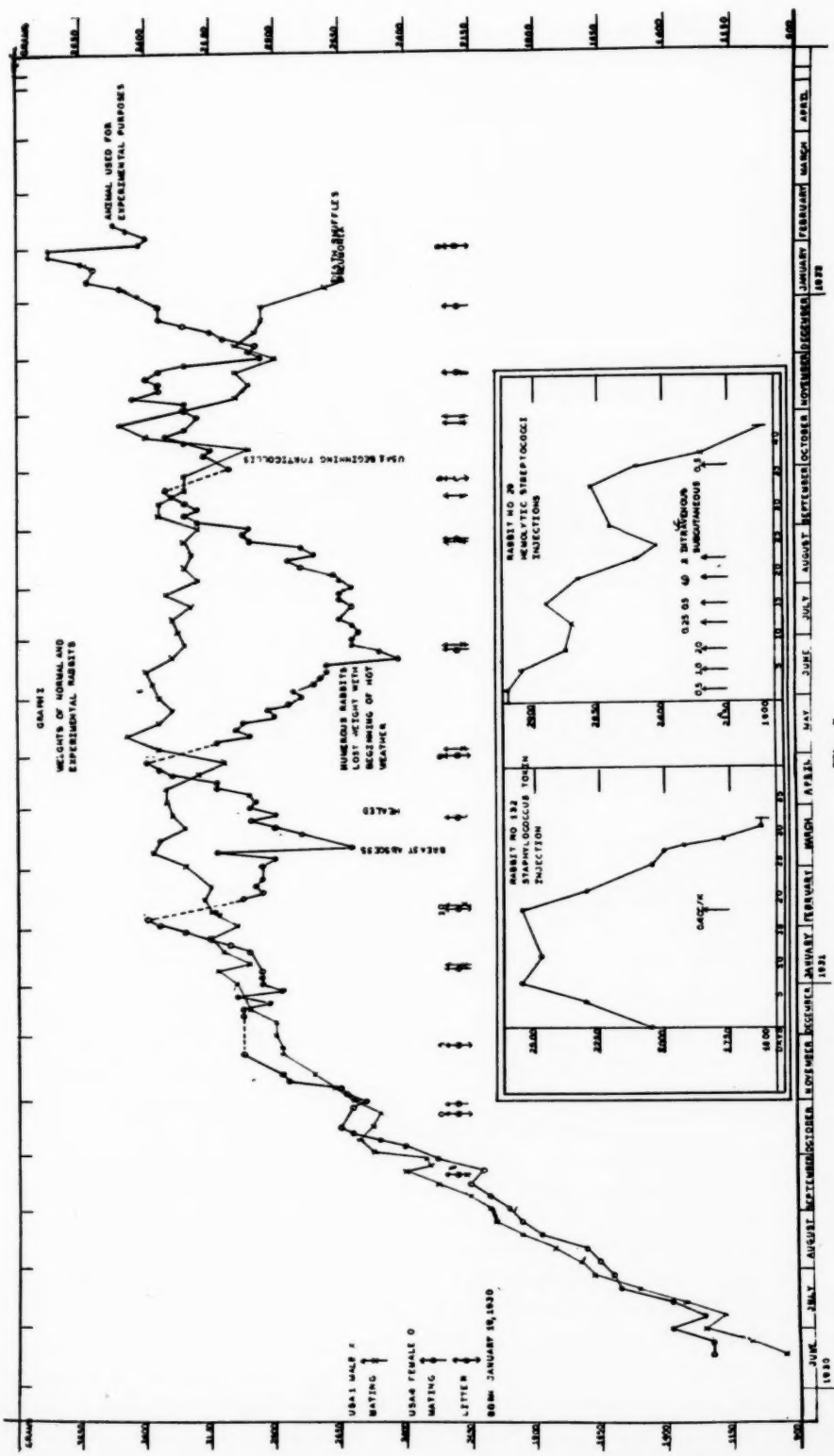
GRAPH 2
WEIGHTS OF NORMAL AND EXPERIMENTAL RABBITS
ANIMAL USED FOR EXPERIMENTAL PURPOSES
USA 4 BECOMING PARTICOLLIS
NUMEROUS RABBITS LOST WEIGHT WITH BEGINNING OF HOT WEATHER
RABBIT NO 132 STAPHYLOCOCCUS TOXIN INJECTION
RABBIT NO 29 HEMOLYTIC STREPTOCOCCI INJECTIONS
USA 1 MALE ♂ MATING
USA 4 FEMALE ♀ MATING
LITTER
BORN JANUARY 16, 1930
JUNE JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER JANUARY FEBRUARY MARCH APRIL MAY JUNE JULY AUGUST SEPTEMBER OCTOBER NOVEMBER DECEMBER JANUARY FEBRUARY MARCH APRIL
1930 1931 1932

Fig. 5.

not be viable. If the young are dead the animal will either abort with a rapid fall in weight or retain the dead fetuses. In the latter case, there will be a slowly falling or stationary weight curve while the dead fetuses are converted into lithopedions or are slowly absorbed.

Following the birth of the young there is a sharp weight loss which is about equal to the previous gain. The normal animal holds this weight and balances its increased metabolism. Following the March, 1931 pregnancy of U5A. 4 there was a marked loss in weight. On examination it was found the animal had a large tumor that involved two breasts. This broke down and drained in a few days. Accompanying the drainage and healing the previous weight loss was rapidly regained.

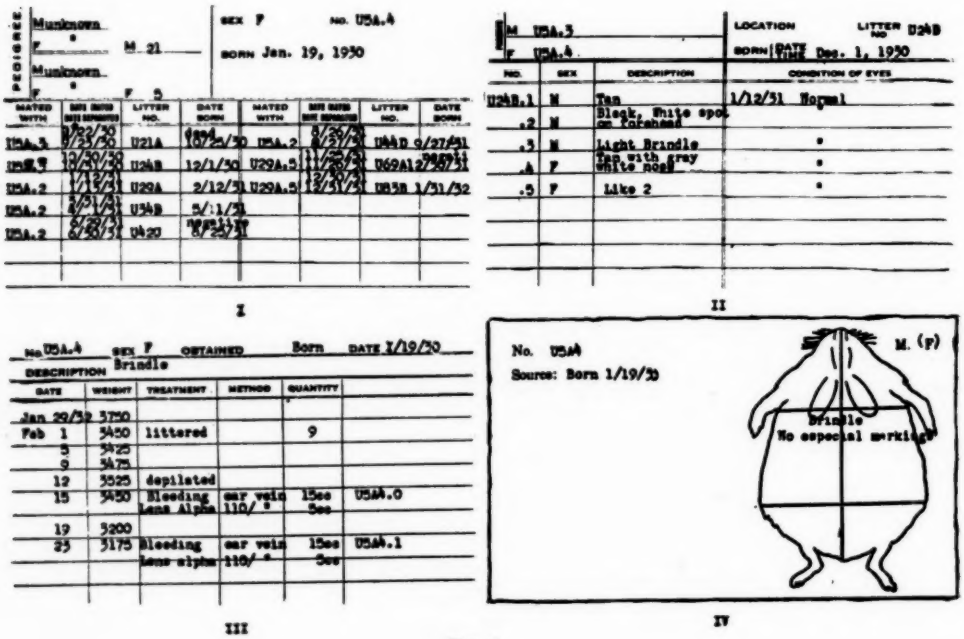

Fig. 6.

With the beginning of warm weather, U5A. 4 while nursing a litter lost considerable weight. This, it is believed, was not due to the nursing burden but was directly associated with the temperature rise as a considerable number of rabbits, male and female, showed a similar loss.

U5A. 4 did not litter during the summer months. In this it follows the general rule. During that summer, although numerous animals were mated no pregnancies resulted. Like U5A. 4 they all had records of previous litters and all littered again during the Autumn.

The weight curve of the male U5A. 1 parallels the female except for the pregnancy changes and the summer weight loss. In the Autumn of 1931 it was noticed that his head was beginning to rotate in the manner characteristic of middle ear infection. Shortly after this he developed a definite nasal discharge with a moderate amount of sneezing. From this time he showed a constant

weight loss and at autopsy a complete consolidation of the lungs was found. The weight loss seemed associated primarily with starvation as the slightest stimulus, auto or external, brought on the barrel rolling associated with torticollis, and he was unable to obtain an adequate amount of food.

The records of rabbits 29 and 132 show the weight changes associated with experimental procedures that may or may not be fatal to the rabbit. These weight changes seem largely dependent upon the general reaction of a rabbit to infection. A sick rabbit is drowsy and lethargic and takes practically no food or water, while a healthy rabbit eats continually. Correlated with this, apart from any destruction of tissue by the toxic substance, the animal with a chronic infection shows a marked loss of abdominal fat at autopsy. For example, rabbit 132, dead two weeks after the injection of staphylococcus toxin showed a complete loss of abdominal fat with no other demonstrable lesions. This toxin is mainly a heart and respiratory poison and after its injection the animal responds with a slowed respiration, marked diarrhea, and a general stuporous state, during which time little or no food is taken. In general, rabbits experimentally infected with organisms or substances lethal for the rabbit show a weight loss that varies with the severity of the induced condition. From the weight curve alone, it is not impossible to predict the death of the rabbit within a few days.

SUMMARY

Methods for the care and breeding of rabbits in the laboratory have been outlined. A detailed record has been presented of the weight changes occurring in normal animals and in a pair of animals injected with substances lethal to the rabbit. From the results, and other data not detailed, it is concluded that a weight record is an excellent guide to the clinical condition of the rabbit.

REFERENCE

1. Pearce, L., and Van Allen, C. M.: Effects of Light on Normal Rabbits With Especial Reference to Organic Reaction. (a.) Clinical and Postmortem Observations. (b.) Organ Weights. (c.) Analysis of Organ Weights, J. Exper. Med. **44**: 447, 461, 483, 1926.

HINTON TEST FOR SYPHILIS

Third Modification*

William A. Hinton, M.D., Boston, Massachusetts

IN the management of syphilis, a growing reliance on serum tests demands continuous research to improve them. As a consequence of research so directed, it became needful to make a third modification of the Hinton test for syphilis.[1, 2, 3] In its modified form as presented here, the accuracy of the test has been increased, the technic has been simplified, and the interpretation is based on a set-up of one tube instead of four tubes for each serum. Though simplified, it requires precision in execution because, as is true of any sensitive method, consistent and accurate results cannot be obtained if minor variations in technic are allowed to creep into the routine.

EQUIPMENT

The equipment required for the test is as follows:

1. Test-tube racks holding twenty to one hundred and sixty tubes. To simplify the numbering and pipetting of the serums, these racks should be constructed to hold ten or twenty tubes in a row.

2. Serum tubes 10 mm. inside diameter and 100 mm. long. The diameter of the tubes should be approximately uniform.

3. A water-bath for heating serums, kept at 55° to 56° C.

4. A Wassermann bath preferably, or an incubator, kept at 37° C. The water in the bath should be changed frequently to prevent a deposit from forming on the outside of the tubes. The Wassermann bath is preferred because by its use the test is from 3 to 5 per cent more sensitive.

5. A centrifuge with which a speed of 2000 revolutions a minute can be obtained.

6. A thermometer that registers maximum and minimum temperatures.

7. Graduated 100-c.c. and 250-c.c. cylinders, for measuring the reagents.

8. Dropping pipettes with rubber bulbs, of about 7.5 c.c. capacity.

9. Serologic pipettes of 1.0 c.c. capacity, graduated in tenths, for measuring the serums, and 5-c.c. or 10-c.c. serologic pipettes for measuring reagents and adding them to the serums.

10. Thick-walled Erlenmeyer flasks[4] of 125 to 500 c.c. capacity, with an inverted V-shaped partition blown in the bottom, for mixing glycerinated indicator (Fig. 1). This partition should produce two semicircular compartments, each of which should hold from 3 to 5 c.c. in flasks with a capacity of 125 or 250 c.c., and from 8 to 10 c.c. in flasks of 500 c.c. capacity. Flasks of this type are not listed in any of the catalogues. I have had mine especially modified for this purpose by Macalaster Bicknell Company of Cambridge, Massachusetts.

*From the Department of Clinical Research of The Boston Dispensary.

PREPARATION OF REAGENTS

The reagents used in the test are: (1) cholesterinized heart extract; (2) 5 per cent salt solution, and (3) 50 per cent glycerol solution.

1. Cholesterinized heart extract. This is prepared as follows: Dried ground beef heart muscle (Bacto-Beef Heart, Dehydrated, Digestive Ferments Company) is extracted by putting 100 gm.* of the powder and 400 c.c. of ether (anesthesia) in a wide-mouthed glass-stoppered bottle and shaking thoroughly by hand for ten minutes. The solid material is allowed to settle for five to ten minutes, and then as much of the ether as possible is poured through filter paper. The ether is discarded. The extracted tissue is scraped from the

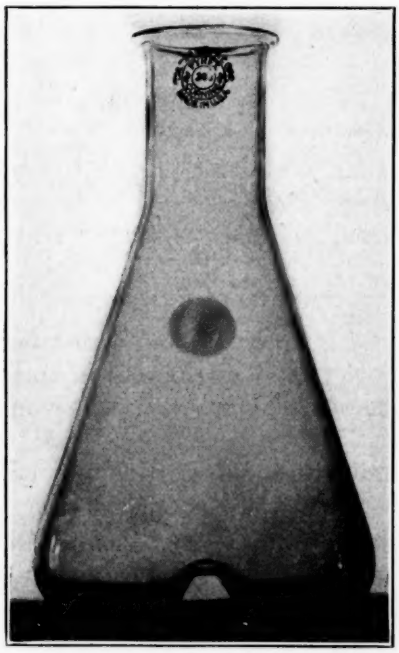

Fig. 1.—Inverted V-shaped partition blown in the bottom of an Erlenmeyer flask.

filter paper and replaced in the bottle for further extractions. A total of five separate extractions must be made with 400 c.c. of fresh ether for each extraction. The main portion of extracted tissue should not be allowed to dry between extractions. A new filter paper should be used for each filtration. After the final extraction, the extracted tissue is dried on the filter paper. This dried residue, which contains the ether-insoluble constituents, is weighed; it is then extracted for three days at room temperature (17° to 20° C.) in a glass-stoppered bottle with 95 per cent ethyl alcohol, in the proportion of 5 c.c. of the alcohol to each gram of residue. The contents of the bottle are shaken vigorously by hand for five minutes, three times each day. The tissue is removed by filtering into a graduated cylinder, the alcoholic extract is measured and transferred to a glass-stoppered bottle, and cholesterol is added to the

*The extraction of larger or smaller amounts of the powder in one operation has appeared to yield an inferior reagent.

extract in the proportion of 0.4 gm. to each 100 c.c. The cholesterol is best dissolved by warming, at 37° C., in an incubator or water-bath, with occasional shaking. (It should not be stored in a refrigerator, for chilling will precipitate the cholesterol; if, by inadvertent chilling, the cholesterol should precipitate, it must be redissolved before use.)

Such cholesterinized heart extracts have been kept in our laboratories in colorless glass-stoppered bottles, at room temperature, for a period of more than two years.

Cholesterol obtained from Merck and from the Digestive Ferments Company has given identical results when compared. All of the many cholesterinized heart extracts prepared in accordance with these directions have given almost precisely the same results.

2. A 5 per cent solution of sodium chloride (C. P.) in sterile* distilled water, to each 4500 c.c. of which 1.0 gm. of salicylic acid (C. P.) should be added. In the paper a solution so prepared will be referred to as 5 per cent salt solution.

The salicylic acid not only helps to preserve the potency of glycerinated indicator (described later), but also minimizes slight changes which occur with some negative serums.

3. A 50 per cent solution of glycerol, prepared by mixing equal volumes of glycerol (C. P.) and sterile distilled water.

The 5 per cent salt solution and the 50 per cent glycerol solution keep indefinitely.

TECHNIC OF THE TEST

1. Centrifuge the blood if necessary, and with a long dropping pipette remove each serum from its clot and deliver into an appropriately labelled serum tube. To avoid contamination of one serum with another, after each serum specimen has been drawn off the dropping pipette should be thoroughly rinsed at least three times with sterilized physiologic salt solution (each time discarding the rinsing solution); and to minimize bacterial contamination, after every twenty serum specimens have been drawn off the washing bottle (about 200 c.c. capacity) should be emptied and filled with fresh salt solution.

2. Heat the serums in the inactivating bath at 55° C. for thirty minutes. Be sure that the level of the water in the bath is above every serum specimen.

Extreme care should be taken to keep the water at 55° C. throughout the entire period of inactivation, as erroneous results may be obtained when the temperature falls even one or two degrees below this point. Inactivation at a temperature above 56° C. decreases the strength of the reactions.

Serums should be inactivated just before testing, as those which may give strongly positive reactions when tested a few hours after removal from the clot occasionally give negative or weak reactions when kept for even as short a time as a day.

Should it be necessary to retest a specimen, always use serum freshly separated from the clot, rather than that which has been inactivated twenty-four hours previously.

3. Set up the racks with one properly numbered serum tube for each specimen to be tested. Be sure that the tubes are clear and clean.

To clean the tubes, rinse them thoroughly, as soon after use as possible, with tap water, and then fill each one with a warm solution of 5 gm. of sodium hydroxide in 1000 c.c. of tap water; allow the tubes to remain immersed in the solution for about two hours,

*No attempt need be made, however, to keep materials or glassware strictly sterile; the purpose is simply to avoid needless gross bacterial contamination which seriously interferes with the accuracy of the test.

and wash *thoroughly* with hot water to remove the alkali. This process usually completely removes, without the use of a test-tube brush, any deposit which may have remained from previous use. (Boiling in this caustic solution corrodes the tubes.) Sterilize in a hot-air oven. This will minimize the possibility of bacterial growth, which may occur in the prolonged incubation. Other glassware should be cleaned in essentially the same manner.

4. With a 1-c.c. pipette graduated in tenths, measure 0.5 c.c. of each heated serum into a separate tube, that has been properly labelled. (Use a separate pipette for each serum.)

It is well to use several strongly positive and several negative serums as controls, particularly when only a few tests are to be made at a time.

5. Compare the appearance of each of the pipetted serums with the appearance of the serum in the tube from which it was taken, in order to be certain that there has been no error in pipetting, or in labelling the tubes.

6. Prepare glycerinated indicator as follows (adhering strictly to the directions for mixing, because incorrect mixing causes unreliable reactions): Pipette one part of the cholesterinized heart extract into one compartment of the Erlenmeyer flask with the inverted V-shaped partition in its bottom, and 0.8 part of the 5 per cent salt solution into the other side.

Great care should be used to avoid admixture of the two solutions when the salt solution is pipetted into the flask. A 125-c.c. flask is suitable for the preparation of 30 c.c. of glycerinated indicator, a 250-c.c. flask for 60 or 90 c.c. of glycerinated indicator, and a 500-c.c. flask is to be preferred for the preparation of larger quantities.

Mix the cholesterinized heart extract and salt solution by shaking the flask rapidly from side to side for one minute. Let the mixture stand for at least five minutes—this is important. Add 13.2 parts of the 5 per cent salt solution and shake thoroughly to obtain complete mixing. Finally, add fifteen parts of the 50 per cent glycerol solution and shake until the suspension is homogeneous. Thirty cubic centimeters of the glycerinated indicator is the smallest amount which can be mixed satisfactorily at one time, and amounts as great as 210 c.c. can be prepared with equal success.

As glycerinated indicator will remain unimpaired in strength for one week if kept in a refrigerator at a temperature of 8° C. or lower, enough may be mixed at a time for a week's use.

7. With a clean 10 c.c. pipette, add, not more than thirty minutes before incubation, 0.5 c.c. of the glycerinated indicator just described to each serum.

8. Pipette 0.5 c.c. of the same indicator and 0.5 c.c. of the 5 per cent salt solution into an empty serum tube. This serves as a control, the purpose of which will be explained later.

9. Incline the rack to an angle of about 45°, and then shake by thrusting it quickly upward and forward, then downward and backward, with sufficient speed to cause the fluid to travel halfway up the tubes. At least three minutes of this motion are required for accurate results. The presence of small bubbles at the surface of the fluid in many of the tubes is the only safe criterion of adequate mixing of the serum with the indicator. This is important.

10. Select the tubes which contain serums that show hemolysis, or cloudiness due to bacterial growth, and test according to the quick method to be described later in the paper.

11. Place the rack of tubes in the Wassermann bath or incubator at 37° C. and let it remain in the Wassermann bath for sixteen hours (conveniently from 5 P.M. to 9 A.M.), or in the incubator for eighteen hours. Do not disturb the contents of the tubes before reading.

12. At the time of removing the tests from the bath or incubator, record the temperature readings shown by the bath or incubator thermometer, and note the readings on the maximum and minimum thermometer. For dependable results the temperature should not fall below 34° C. nor rise above 39° C.

READING THE TESTS

To read the tests, sit in front of a window, but not in the direct sunlight. (The light must be good, and for this reason good artificial light must be provided for reading on dark days or at night.) Lift each tube carefully from the rack, and holding it at the level of the eye, look at it in the direction of a darkened background on either side of a window or of a suitably placed light. Look for any clearing of the fluid and for a significant* ring or band at the top of the meniscus of the fluid. If such a ring is not seen with the tube in this position, incline it to an angle of 45° and observe further. At the same angle, gently rotate the tube by rolling it between the fingers, and again look for a ring. Finally, the tube is shaken to detect even the faintest precipitate.

INTERPRETATION OF TESTS

The reactions are designated as positive, negative, doubtful, and unsatisfactory, without indicating the intensity of the change in positive reactions.

The simplicity of this method of recording and reporting has the advantage of not confusing physicians by implying that the test is in any way a quantitative reaction. After a careful study, I find that, as in the Wassermann test, the intensity of the reaction of this test bears no relation to the clinical condition of the patient.

Positive Reactions (recorded +).—A ring or band of coarse granules or flakes of lipoids, approximately 0.2 mm. to 1.5 mm. wide, slightly to moderately, but not strongly adherent to the walls of the tube, is present at or a little above the level of the meniscus. This ring or band is accompanied by complete clearing of the fluid, except for large agglutinated masses of lipoids, or by partial clearing with only slight agglutination. Gentle shaking causes the ring or band to disperse and the particles to become visible as a definite precipitate throughout the fluid. In rare instances there is no ring, but a fine precipitate becomes visible on shaking. A positive reaction, if definite, is valid even though the serum shows evidence of bacterial contamination or hemolysis.

Negative Reactions (recorded –).—There is no clearing, no ring, no band, no precipitate—in fact, there is no visible change.

Unsatisfactory Reactions (recorded "Unsat.").—The reaction is designated as unsatisfactory (except when it is definitely positive) whenever the blood or its serum has partially decomposed. This decomposition visibly manifests itself by hemolysis, and by cloudiness due to bacterial growth. In the test, hemolyzed serums frequently produce a whitish ring, which differs from the

*To be significant, a ring must be only slightly adherent to the wall of the tube, and must possess coarse granules or fine flakes which have a tendency to "creep" up the wall of the tube in a thin film when the tube is gently shaken.

significant ring found in positive reactions in that it sticks to the tube even after vigorous shaking. In the event of bacterial contamination, there is a filmy white aggregation of lipoids at the top of the fluid, which on first observation may be confused with a positive reaction. With only a little experience, however, one can readily distinguish between the coarse granules or the flakes of a positive reaction and the fine filmy cloud due to contaminated serum. If a serum shows hemolysis (as manifested by redness greater than that produced by a solution of 0.1 c.c. of blood in 3 c.c. of distilled water), or if it shows evidence of bacterial contamination at the time of reading, the reaction is designated as unsatisfactory. This interpretation should be made because even the moderately hemolyzed or bacterially contaminated serum of a known syphilitic will very often give a negative reaction, and because, as will be shown later, a negative reaction with a proper serum has great accuracy in excluding syphilis.

Doubtful Reactions (recorded ±).—If only a slight granularity is seen on shaking, beyond that observed in the control tube (containing 0.5 c.c. of indicator and 0.5 c.c. of the 5 per cent salt solution), or if only a slightly flaky or slightly granular ring is seen, the interpretation must be guarded. Centrifuge for ten minutes, at high speed (about 2000 revolutions a minute) the tubes which show such reactions. Also centrifuge all tubes having serums that are somewhat opaque in themselves, but are not opaque because of bacterial contamination or hemolysis, since opacity may mask a doubtful reaction. It is important to restrict the use of the centrifuge to this condition and to the quick method to be described; otherwise, doubtful reactions will very frequently occur with the serums of nonsyphilitic persons. Centrifuging, as indicated by Eagle,[5] causes the particles of an indefinite precipitate to cohere so as to be easily visible. The reaction is considered doubtful if at the meniscus a thin film of lipoids is formed which, on shaking, breaks up into fine flakes or coarse granules that persist as a precipitate even after vigorous shaking. When centrifuging does not markedly intensify the precipitate, the reaction is called negative.

A QUICK METHOD THAT DETECTS MOST OF THE POSITIVE REACTIONS

The quick method to be described is advantageous in two types of cases:

1. Whenever there is immediate need for a report the routine procedure should be carried out up to the point of incubation; but before incubation the tests in question should be centrifuged at high speed (about 2000 revolutions a minute) for ten minutes. Immediately after centrifuging, the readings should be made. Plainly visible clearing and a well-marked precipitate, seen on shaking, denote a positive reaction. On the other hand, the presence of only a very fine precipitate visible on shaking indicates no more than a probable positive reaction. The absence of a precipitate is reasonable but not conclusive evidence of a negative reaction. The tubes that show a probable positive reaction or a negative reaction should be well shaken and then placed in the water-bath for sixteen hours, or in the incubator for eighteen hours, after which the reading and the interpretation are made as if the tests had been conducted in the routine manner.

2. Specimens that are hemolyzed or bacterially contaminated should be tested by the quick method to avoid their further deterioration. In reporting the results of such tests, it is important to know that, in proportion to the degree of deterioration, either hemolysis or bacterial contamination tends to prevent a positive Hinton reaction with serum from a syphilitic person. Therefore, tests that show a positive reaction with such serums should be so reported, but all others should be reported as unsatisfactory, without further incubation.

SIGNIFICANCE OF THE TEST

A positive Hinton reaction almost always indicates syphilis. Nevertheless, a single positive reaction must not be the basis for a diagnosis of syphilis unless supported by definite clinical evidence of the disease. Whenever a single positive reaction is unaccompanied by definite clinical signs of syphilis, checks should be made by tests on at least two additional specimens of blood before concluding that the positive reaction is dependable. If the test is used in this way, practically no incorrect diagnoses of syphilis will be made. I have noticed particularly that neither rheumatic fever nor pneumonia appears to cause, with the Hinton test, false positive reactions, as are so frequently encountered with the Wassermann test. As a guide during treatment, a positive reaction usually indicates that continuation of the treatment is necessary to prevent relapse. In cases where prolonged intensive treatment has not caused the positive reaction to disappear, however, there is a question as to the significance of this unyielding ("persistent") positive Hinton reaction. I do not feel that the test has been in use long enough to offer an answer to this question. I do believe, however, that continuous clinical observation is necessary in all such cases, and that most of them should have some type of antisyphilitic treatment as well. My own experience, as well as that of others,[6] shows that during intensive treatment positive Hinton reactions almost always persist a third longer than positive Wassermann reactions, and in many instances more than twice as long. Inasmuch as negative Wassermann reactions often occur prematurely during treatment, as demonstrated by frequent serologic or clinical relapses, or both, the persistence of a positive Hinton reaction would indicate a superiority of the Hinton test as an aid in the management of syphilis, especially in cases where modern treatment is begun during the first weeks or months of the disease.

A doubtful Hinton reaction suggests syphilis, and occurs for the most part in patients in the beginning of the primary stage, or in syphilitics who, serologically, have responded almost completely to treatment. In my experience the doubtful reaction has occurred in from 5 to 15 per cent of persons in whom syphilis was excluded with reasonable certainty.

A negative Hinton reaction is seldom erroneous, except during the first few days of the primary stage. Nevertheless, a single negative Hinton reaction alone is insufficient reason for excluding syphilis. In a study of 161 cases of known syphilis of three years' or less duration, in which, during treatment, 929 Hinton tests (counting all, from the first positive to the last positive, inclusive) were made, there occurred but 6 per cent (54) false negative reactions. In this same group, three or more consecutive negative reactions followed by positives occurred in only 3 per cent (5) of the cases. Thus, even three negative

reactions are not necessarily decisive evidence of cure of a syphilitic person, though such a finding seems to be the best available evidence of permanent relief, aside from continued clinical observation. A basis for this belief arises from the fact that of the patients studied, 90 per cent of those who had three or more consecutive negative reactions had shown neither clinical nor serologic signs of relapse.

REFERENCES

1. Hinton, W. A.: A Glycerol-Cholesterol Precipitation Reaction in Syphilis, Boston M. & S. J. **196**: 993, 1927.
2. Hinton, W. A., and Stuart, Genevieve O.: The Hinton Glycerol-Cholesterol Agglutination Reaction; Modifications in Technic (Second Communication), J. Lab. & Clin. Med. **14**: 621, 1929.
3. Hinton, W. A., and Berk, Arthur: The Hinton Glycerol-Cholesterol Reaction for Syphilis. Second Modification, New England J. Med. **202**: 1054, 1930.
4. Hinton, W. A.: A Flask for Diluting Antigens Used in Serum-Tests for Syphilis, Am. J. Clin. Path. (In press.)
5. Eagle, H.: Studies in the Serology of Syphilis, Am. J. Syphilis **16**: 218, 1932.
6. Sanford, A. H.: Which Test for Syphilis? Am. J. Clin. Path. **1**: 347, 1931.

A PRACTICAL TECHNIC FOR BLOOD CULTURES*

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THE demands for varied bacteriologic technic in a large general hospital render the simplest procedures the most expedient. Blood culture technic in particular would seem to require the least possible handling of inoculum so as to allow for minimal chance of contamination and maximum accuracy of findings. The following procedure has been found to give consistent results particularly for cases of endocarditis and pneumonia.

Technic.—About 20 c.c. of blood is withdrawn from the median basilic vein under sterile precautions and added to 10 c.c. of citrated saline (2 per cent sodium citrate in 0.85 per cent saline). The tube of blood is allowed to stand until a good portion of the red blood cells have settled. Pipette about 5 c.c. of the settled blood cells and transfer equal portions to two tubes of melted agar (temperature 45° C.). Mix well and pour plates. The second plate will receive the heaviest concentration of red blood cells and the most bacteria. Transfer 15 to 20 c.c. of hormone broth, reaction 6.8 to 7.2, to the test tube in which the blood was collected. This tube will contain the major portion of the plasma and the residue of blood cells. Large potato tubes containing 15 to 20 c.c. of hormone broth are prepared. The final broth culture usually approximates 35 c.c. in volume. Incubate at 33° to 36°.

The plates and tubes are observed daily for the first three or four days, but are not opened unless positive culture is indicated. On the third or fourth day a sample of culture is removed from the depth of the broth and centrifugalized preparatory to the examination by Gram's technic. A transfer for verification by subculture is generally made at this time. Samples from the sediment are

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examined in like manner on the seventh, fourteenth, and twenty-first days and negative cultures are not discarded until the third or fourth week. The plates may be discarded at the end of the first week.

Certain theoretical considerations which have been borne out by findings have influenced the employment of the above procedure. Of prime importance is the dilution of bactericidal content of the blood serum by the sodium citrate solution. Next in importance is the fact that nonmotile bacteria may be held to the blood cells as colloidal particles and would settle accordingly. It would be expected that delicate organisms, few in number, might be protected by the sediment of blood cells during the period of lag.

Gross Findings.—In many instances where plates have been negative, organisms have been found in profuse numbers in the blood cell sediment. Pneumococci and enterococci have been found to cloud supernatant broth faintly and the former can be typed by means of the precipitation method of Krumwiede and Valentine. Enterococci may be identified by means of positive aesculin fermentation and negative methyl red test.

Meningococci and gonococci grow profusely in the depth of the red blood cells, growth starting upon the individual cells and continuing as a gelatinous semiopaque ring at the meniscus of the broth after the bottom layers of culture have been disturbed. Meningococci are more resistant to heat when protected by the sediment of red blood cells.

Micrococcus albus, when present even in small numbers, grows in the layer of white blood cells at the bottom of the tube and continues to grow as a slimy white ring with trailing masses at the meniscus of the broth after the substratum of blood cells has been disturbed. Staphylococci generally cloud the supernatant with discrete accumulations of growth and produce a granular pellicle. Diphtheroid bacilli grow in a fine white layer above the layer of white blood cells in the substratum. Cases of septicemia approaching a fatal termination give equally profuse growth of organisms in the supernatant and substratum of broth cultures as well as in the plates. A profuse growth in the broth cultures from cases of epidemic pneumonia does not necessarily indicate unfavorable prognosis. Higher bacteria grow as round, fluffy, mold-like masses about the size of a pea in the layer of white blood cells or adherent to the wall of the tube. In blood agar plates colonies of them appear morphologically like Streptococcus viridans. Cases of bacterial endocarditis in which plates and supernatant broth appear grossly negative frequently yield profuse growth of organisms in the red blood cell substratum. Subcultures of the same on blood agar slants are so delicate as to seem negative.

Conclusion.—A practical blood culture technic is described which provides for the isolation of delicate organisms in blood agar and broth by protecting them in a substratum of red blood cells.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

B. C. G. VACCINE, Leukocytic Changes in Vaccinated Children, Dolgopol, V. B., and Stitt, R. H. Am. Rev. Tuberc. 26: 304, 1932.

The blood picture of 37 children vaccinated with the Bacillus Calmette-Guerin was examined at frequent intervals. The time of observation varied from one to thirteen months after vaccination.

The blood of 5 normal babies was examined, at similar intervals, for a period from four to seven weeks.

The fluctuations of the number of monocytes and lymphocytes that were observed in the vaccinated children were within the normal range of variations for the young children.

The fluctuations of the number of monocytes and lymphocytes were greatest in the newborn babies (the group of children vaccinated by the oral method); the older children (those vaccinated subcutaneously); showed the least changes in the blood picture.

An increase in the number of monocytes was often observed on the day of intracutaneous tuberculin test, or on the day following the test, irrespective of a positive or negative reaction to tuberculin. The increase in the number of monocytes after tuberculin tests was seen also in some children before the vaccination (probably a response to some nonspecific factor).

Nine cases vaccinated by parenteral routes showed a slight elevation of the number of monocytes between the first and the third day after vaccination.

A steady relatively high monocyte count and a low lymphocyte count, without any intercurrent infection, were observed in one case (oral vaccination), or in 2.7 per cent of all cases; after four months the blood picture of this child became normal. The child was well after two years of clinical observation.

A complete autopsy on one child that died of enteritis and pneumonia did not reveal any tuberculosis or tubercle-like lesions in the organs or in the lymph nodes; this child shows a high monocyte and lymphocyte count both before and after vaccination (oral route).

The slightest degree of hematologic changes after administration of the Bacillus Calmette-Guerin, especially infrequency of a noticeable increase in the number of monocytes, indicates low tuberculogenic properties of this bacillus.

The hematologic response to the vaccination with the Bacillus Calmette-Guerin, as expressed in the fluctuations of the number of monocytes and of lymphocytes, is not great. The blood picture cannot therefore, be used as an index of the process of immunization with the Bacillus Calmette-Guerin, as proposed by Pittaluga and Garcia.

SEDIMENTATION TEST, in Pulmonary Tuberculosis, Kaminsky, J., and Davidson, D. Am. Rev. Tuberc. 26: 282, 1932.

The results of the red-cell sedimentation test in a series of 500 cases are reported.

No definite relationship could be established between variations in the number of red cells and the sedimentation speed. (Red-cell counts and sedimentation tests were done in a group of 92 cases and the number of red cells ranged from 4,150,000 to 5,990,000.) A low color index was found to accompany a rapid sedimentation velocity.

In 48 normal individuals the sedimentation index ranged from 1¾ to 10¼, with an average of 4.5 for the men, and from 5¾ to 11¾, with an average of 9.15 for the women.

There is a close parallelism between activity of the lesion and the sedimentation rate in pulmonary tuberculosis. The group of patients, whose sedimentation graphs were hori-

zontal lines, showed the lowest incidence of positive sputa, cavity-formation; pulse and temperature elevations and substandard weight. The group of patients whose sedimentation-test graphs were vertical curves, showed the highest incidence of objective signs of activity.

There were no cases with active pulmonary tuberculosis showing normal sedimentation values on repeated tests. There was one instance in which a seemingly active case showed a normal sedimentation graph, but the graphs obtained in 5 subsequent sedimentation tests done during the following eight months were all abnormal.

In four patients who died at the institution, the sedimentation tests done within a month before death showed slower sinking velocities than the tests taken in the preceding months.

The sedimentation test is of considerable value as an indicator of activity. A single determination may be of some help in appraising a case of pulmonary tuberculosis, and repeated tests may furnish much additional information as to the progress of the case and serve to estimate effectiveness of treatment.

ARTHRITIS, Correlation of Experimental Streptococcic Arthritis in Rabbits With Chronic Rheumatoid Arthritis, Hadjopoulas, L. G., and Burbank, R. J. Bone and Joint Surg. 14: 471, 1932.

By incubating the whole joint the authors were able to demonstrate these infecting organisms in practically all lesions associated with chronic atrophic arthropathies. Streptococci were found in every part of the synovial tissues where pathologic changes could be detected. No streptococci could be demonstrated in compact bone, yet the spread of infection in bone tissue could be traced step by step through its medullary and haversian vascular supply. In the avascular cartilage the spread of streptococci was through infected bone tissue and synovial infiltration. Normal joints were subjected to the same procedure with uniformly negative findings.

To complete the picture of infection, the authors have recently studied the tendons and muscles in the immediate neighborhood of arthritic joints. We had no difficulty in demonstrating streptococci in tendons at the zone of nuclear proliferation. Muscles were extensively infiltrated with streptococci at their sheaths but in spite of great care the possibility of outside contamination in surface areas must be considered.

Throughout this study the progress of the disease could always be traced directly to the terminal blood vessels as is true in all infections of hematogenous origin. Despite the specific selectivity of the arthrotropic streptococci used in these experiments, internal organs, especially the liver and kidneys, were not exempt from pathologic changes of toxic nature involving their parenchyma. Nevertheless, streptococci could be demonstrated in the interlobular connective tissue of the liver and occasionally even in the hepatic vessels. This finding is highly significant in that it forcibly brings home the fact that chronic rheumatoid arthritis is fundamentally a systemic disease, and it explains the transient septicemias that occur intermittently in the course of the infection.

MENINGOCOCCUS, Specific Precipitin Reaction Associated With Growth on Agar Plates, Petrie, G. Brit. J. Exper. Path. 13: 280, 1932.

Characteristic haloes have been observed round colonies of "smooth" strains of meningococcus, pneumococcus and B. dysenteriae (Shiga), when these bacteria were grown on agar plates which contained the homologous immune-serum.

The haloes consist of a precipitate which is formed by the interaction of the specific polysaccharide with the homologous antibody.

A "rough" strain of pneumococcus, by virtue of the "rough" polysaccharide or the somatic nucleoprotein, forms haloes in the presence of a "rough" or a type-specific serum; the haloes round "rough" colonies are extremely tenuous.

The haloes that are produced by the meningococcus and by both "smooth" and "rough" variants of the pneumococcus represent in the initial stage a uniform deposit of the precipitate; at a latter stage the precipitate tends to assume the form of Liesegang rings.

When the specific polysaccharide which has been isolated by Morgan from B. dysenteriae

(Shiga) is deposited in suitable concentration upon an immune-serum agar plate, the resulting precipitate takes the form of a typical halo with concentric zones.

The ability to form specific haloes constitutes a test for distinguishing between the "rough" and "smooth" variants of bacteria.

The technic follows:

Agar plates were prepared as follows: (1) With the addition of 2.5 per cent sodium chloride; (2) with 5 per cent normal horse-serum; (3) the same as (2), but with 2.5 per cent sodium chloride; (4) with 5 per cent antimeningococcus serum (Type I); and (5) the same as (4), but with 2.5 per cent sodium chloride. The salt was added with the idea that it might intensify the "rough" appearance of colonies that were composed of salt-sensitive cocci. The anti-meningococcic serum was a mixture from four horses which were being immunized with Type I cultures; the mean agglutination titer was 1 in 450. Agar plates prepared in this way were inoculated with each of the two strains.

Three days later a delicate halo was seen round the colonies of the recent strain on the agar to which antimeningococcus serum had been added, but not round those of the old laboratory strain. The haloes were apparent on the plates, both with and without 2.5 per cent sodium chloride.

These observations, which are supported by further work, indicate that the halo represents a specific precipitate which is formed by the interaction of the antibody in the serum with the specific polysaccharide of the meningococcus; this is set free from the cocci in the colony by a process of secretion or autolysis, and diffuses into the agar round the colony.

B. TYPHOSUS, Value of Wilson and Blair's Bismuth Sulphite Medium for Isolation of, From Feces and Sewage. Stewart, A. D., and Ghosal, S. C. Ind. J. M. Res. **20**: 341, 1932.

The authors found this medium much superior to McConkey's. The formula follows:

To 100 c.c. of melted 3 per cent agar are added 5 c.c. of a 20 per cent solution of glucose, 10 c.c. of a 40 per cent solution of sodium sulphite (anhydrous), and 5 c.c. of a standard bismuth solution. After boiling for two minutes an addition is made of 1 gm. exsiccated sodium phosphate dissolved in 10 c.c. of boiling water and 1 c.c. of 8 per cent solution of ferrous sulphate crystals. To it is then added 0.5 c.c. of a 1 per cent watery solution of brilliant green and 2 c.c. of absolute alcohol.

The standard liquor bismuthi is prepared by mixing 60 gm. of bismuth citrate with 50 c.c. of distilled water and then 20 c.c. of liquor ammoniae of specific gravity 0.880 and finally making the volume up to 500 c.c. with distilled water.

It has been stated by some that the blackness of the colonies depends upon the brand of bismuth citrate used; but different brands have given equally good results in the author's hands.

It should also be noted that the specific gravity of the liquor ammoniae as advocated by Wilson and Blair should be strictly 0.880; any deviation from this gives indifferent results.

BLOOD: Studies on Blood and Tissue Reactions, Harvey, W. F., and Hamilton, T. D. Edinburgh M. J. **39**: 285, 1932.

I. Notes on Technic:

This paper, which because of its detail does not lend itself well to abstraction, is referred to because of the many practical suggestions, based on experience, which it presents. Those interested in hematologic methods will be well repaid by its perusal.

II. The Differential Blood Count:

The following conclusions are advanced:

There is not a very large error in making a single leucocyte total count provided that the necessary precautions are taken.

The differential cell-count on a well-made, and to a surprising extent on an ill-made, film is significantly valid for diagnostic purposes.

There is evidence of long-wave diurnal oscillation of leucocyte numbers and proportions, but not sufficient evidence of a superimposed periodic short-wave type of fluctuation.

All sorts of factors, such as sleep, posture, heat and cold, muscular exercise, locality of examination, digestion and simple ingestion, may be regarded as traumata and may produce leucocytic variation of great or less duration and may combine together to enhance or to mask the variation due to any one factor singly.

The authors emphasize the importance of a properly made smear of such a size that the entire area of it may be counted without undue labor.

III. The Peripheral Blood Picture Is the Image of the Focal Lesion.

That the peritoneal fluid is closely related to blood fluid is a reasonable supposition. That it should, when debris, dead cells, desquamated cells and reparative cells or structures are excluded from consideration, contain more or less the same cells as the blood is only what one would expect. This investigation therefore, in so far as it affirms a close correspondence in the relative proportions of these cells brings out the fact that the blood fluid in, and in the vicinity of, a disease focus has a similar cell composition and the same significance for the phase of the disease as has the peripheral circulating blood fluid. It is also contended that the peritoneal cavity is a lymph space or tissue space quite comparable with those of lesser dimensions elsewhere and that introduction of an irritant into the peritoneal cavity is to be compared with its introduction elsewhere in the body and to a traumatism. Again, it is only to be expected that, with a complex stimulus to cell exudate, such as may be supplied by a pathogenic organism continuing to multiply and perhaps distributed in several foci not all in the same stage of development, we should have a peripheral blood picture representing the superposition of a number of phases of focal reaction. This however does not negative the contention that the blood picture closely resembles, so far as its particular types of cells go, that of the focal disease from which it is concluded that the peripheral blood picture is the image of the focal lesion, whatever be the nature of the irritant.

VACCINES, Clinical Reactions to, as Guides to Treatment, Thomas, W. S., and Touart, M. D. Am. J. M. Sc. **184**: 240, 1932.

It is recognized, or should be, that the dose of bacterial vaccines is determined by the reaction produced in the patient and the authors propose, therefore, methods whereby the reactivity of the patient to the vaccine may be determined as a guide to their administration.

Early Local (Wheal) Reaction.—The early positive reaction at the site of injection of a vaccine is a wheal at least 0.5 cm. larger in diameter than any which may appear at the site of the control injection. It frequently has an irregular outline or even pseudopods and is often surrounded by a zone of erythema (areola). Itching may be present. The wheal reaction is at its height in from 10 to 30 minutes and fades soon thereafter.

Significance of the Early Local (Wheal) Reaction.—Occurrence of the early wheal reaction is taken to indicate that the vaccine causing it should be used in the treatment of the case. This reaction is of aid in determining subsequent dosage of the vaccine. Should the reaction be very marked, the treatment dose next following is not increased, other things being equal.

In order to record the wheal reaction the circumference of the wheal and of the areola are outlined upon the skin with pen and ink and numbered. When dry, the picture is transferred by contact to a sheet of damp white blotting paper and this, when dry, is filed for record.

Late Local Skin Reaction.—The late positive reaction at the site of inoculation is not unlike the Schick reaction in appearance, but in order to observe it properly its component features are noted by touch as well as by sight and with the assistance of the patient's sensations as described in response to questioning. The sum total of the symptoms are recorded graphically as they appear, change and disappear during the days following the test. Any or all of the following symptoms have been observed at the testing site:

(1) An indurated nodule, slightly or not at all elevated above the surrounding surface of the skin and about 0.5 cm. in diameter or larger, sometimes lasting for several or even for many days. Long endurance of it has come to be looked upon as a favorable prognostic sign if the vaccine treatment can be thoroughly carried out. (2) Redness of the skin over the nodule. (3) A surrounding zone of erythema (areola) from 1 to 10 or more centimeters in

average diameter. (4) Tenderness on pressure, usually at the nodule only, but sometimes extending over the whole of an existing areola. (5) Heat at the injection site or over the whole of the areola, usually beginning in from six to twelve hours and lasting for one or two days. (6) Swelling of the whole areola. (7) Lymphangitis, beginning in from eight to twelve hours and lasting for a day or two, has been observed in about 2 per cent of the patients who showed late local reactions. (8) Pustule or vesicle in the nodule. This is not due to infection, as repeated culturings have always proved sterile. (9) Pigmentation of the surface of the nodule occurs late and is not often seen. (10) Desquamation of the skin over the site of the disappeared nodule is not common but occurs so late that it has doubtless been overlooked at times. Very rarely a slough has occurred at the site of the inoculation.

The features of the late focal reaction may be recorded upon a graph in which N denotes a nodule 0.5 cm. in diameter; NN or N2 a nodule twice as large. Redness of the nodule is denoted by the letter R; T indicates tenderness on pressure. The findings of the late local reaction are read and recorded upon at least two successive days following the test. A late reaction is considered to be positive when, in addition to any features occurring at the control site, there are present during 2 or 3 days, three or more of the component features named above. Such reactions as vesicles and ecchymoses are considered to be positive even though occurring alone, and to indicate that the organisms causing them should be used in vaccine treatment of the case.

Significance of the Late Local (Skin) Reaction.—A positive late local reaction is taken to indicate that the autogenous vaccine causing it should be used in the treatment of the case. A very marked late local reaction calls for a smaller amount of vaccine to be given in the next following therapeutic dose than does a slight reaction. During the course of a series of treatment injections each late skin reaction helps the operator to determine the amount and the time of the next vaccine dose.

General Toxic Reaction.—This term has been selected to express the rather frequently seen symptom-complex following vaccine injections and characterized by malaise, nausea, drowsiness, feverishness and occasionally swollen and tender lymph nodes. Any or all of these symptoms may occur within from four to twelve hours after the administration of the vaccine. This reaction occurs only in conjunction with a positive late local reaction.

It signifies that an overdose of vaccine has been administered. It does not appear to furnish evidence for or against a specific action of the vaccine causing it, but it is of aid in determining subsequent vaccine dosage.

Symptomatic Reaction.—This name is applied to the onset or increase of allergic symptoms such as bronchospasm, urticaria, joint pains, etc., following within forty-eight hours after the administration of vaccine. This reaction occurs only in conjunction with a positive local reaction.

Relief Reaction (Temporary).—In 45 of the patients quick relief followed the testing or other injection of vaccines. The relief obtained was either partial or complete and occurred within a few minutes or hours after the vaccine injection and lasted for hours or days or even longer.

This reaction appears to indicate that a favorable dose of a specific desensitizing substance has been administered. It is therefore an aid in fixing upon subsequent dosage.

Focal Reaction.—By this term it is intended to signify a lighting-up of symptoms of infection at the site from which the vaccine organisms were obtained. It appears, if at all, within from four to twelve hours after administration of the vaccine.

A focal reaction is thought to indicate that the vaccine causing it is specifically related to a focus of infection but is not necessarily connected with the allergic or other symptoms that are under treatment. It may indicate the need for focal surgery at a site hitherto unsuspected.

Recurrent Local Reaction.—This phenomenon consists of the reappearance of a faded late skin reaction, days or weeks after its disappearance. It follows in from six to twenty-four hours after the reinjection of a vaccine elsewhere in the body.

At present this reaction does not appear to afford information that may aid in planning subsequent vaccine treatment.

Negative Reaction.—This term means, of course, the absence of local and constitutional effects from the injection of a vaccine.

The absence of all reactions appears to indicate that the organisms used are harmless to their host and that they will be of no benefit in treatment and should not be so used.

The plea is made for the adoption of certain standard procedures in all cases in which the employment of vaccine therapy is contemplated, namely:

1. Careful selection of patients, so as to avoid attempting vaccine therapy in persons unfitted for the method.

2. A complete bacteriologic survey of the patients and the preparation of standardized vaccines from all the organisms recovered, except those that are spore bearers.

3. The performance of skin testing with all vaccines so obtained.

4. Careful observation, recording and interpretation of the reactions that follow such tests.

5. Application of the data thus obtained to the subsequent management of the cases.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Fungous Diseases*

A COMPREHENSIVE discussion of molds responsible for disease and the pathologic changes consequent to their activity. For further review notes see editorial in this issue.

Hautkrankheiten und Ernahrung**

URBACH'S volume on Diseases of the Skin and Nutrition With Special Reference to Dermatoses of Childhood, discusses the dietetic treatment of skin diseases. Dermatologists in general are giving up the idea that most dermatologic lesions are purely local and are interpreting them in terms of general metabolism. This volume represents a distinct forward step in this respect.

The first or general portion discusses the influence of nutrition on the chemistry of the skin and on its biologic reactions. Then follows a chapter on nutritional changes as causes for cutaneous manifestations. This includes such conditions as the vitamin deficiencies, dehydration, general undernutrition, milk sickness, and the like. Next follows a chapter on dermatologic lesions associated with alimentary tract infections and intoxications; one on dermatoses due to functional disturbance of the digestive organs. The sixth chapter deals with dermatoses as causes for metabolic disturbances of internal organs and the seventh discusses food idiosyncrasy.

Since Urbach, following the work that was inaugurated by Luithlen, has developed a special method of allergic therapy for the dermatoses, this section is of unusual interest.

The author prepares peptones of the various foods commonly used, and feeds his allergic patient a specific peptone for each of the food articles which the patient is going to receive at the next meal. In this manner he believes he accomplishes specific alimentary desensitization prior to the meal. The method is described in detail and accompanied by the presentation of several illustrative cases.

In the second section the specific dermatologic diseases are taken up alphabetically. The dietary principles of therapy in each case are described in detail.

Lang's German-English Medical Dictionary†

THIS volume, in its fourth edition, contains 56,500 definitions. Only scientific and medical terms are included. This is not a general German English Dictionary. The common words of everyday usage may be found in the ordinary German English Dictionary. This distinction between a general German English Dictionary and this Medical German English Dictionary corresponds to the distinction between Webster and Dorland in our own language.

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*Fungous Diseases: A Clinical and Mycological Text. By Harry P. Jacobson, M.D., Attending Dermatologist and Member of the Malignancy Board of the Los Angeles County General Hospital, Thomas, Springfield, Ill., 1932, pp. 317, cloth.

**Hautkrankheiten und Ernahrung. mit Berucksichtigung der Dermatosen des Kindesalters. Von Dr. Erich Urbach. Privatdozent fur Dermatologie an der Universitat Wien I. Assistent der Universitatsklinik fur Geschlechts und Hautkrankheiten in Wien (Vorstand Prof. Dr. W. Kerl) Mit 55 Abbildungen, 8 Kurven und 10 Tabellen. Cloth. Pages 260. Wien, Verlag von Wilhelm Maudrich, 1932.

†Lang's German-English Dictionary of Terms used in Medicine and the Allied Sciences with their pronunciation. Revised and Edited by Milton K. Meyers, M.D. Neurologist to the Northern Liberties Hospital; Chief of Nerve Clinic, St. Agnes Hospital; Consulting Neurologist, Jewish Hospital, Philadelphia, etc. Fourth Edition Enlarged. Cloth. Pages 926. P. Blakiston's Son & Co., Inc., Philadelphia.

Those who, with a hobbling acquaintance with German, have tried to dig out their own scientific translations will appreciate this volume. With its use, the German compound words will not be quite as horrifying.

In the fourth edition, the editor has added a key to pronunciation for each word.

Medical and Surgical Year-Book*

DURING the summer of 1929 the Physicians' Hospital of Plattsburgh undertook a period of undergraduate instruction for a limited number of medical students between their third and fourth years, major attention being given to cardiac disease. Lectures and clinics were given both by the local members of the Hospital and by invited guests from several of the medical centers. These papers were then collected and have been published in book form. In this form they become a very readable and very excellent symposium on cardiology.

The summer courses in cardionephritis for undergraduate students in medicine will be continued from year to year.

Clinical Interpretation of Laboratory Reports**

A VERY comprehensive volume on the interpretation that may be made by the clinician of reports rendered by the laboratory. The information detailed is as brief as is consistent with the objective of the book. The volume will therefore have greatest appeal to the general practitioner rather than to the internist who would often wish to find more detail.

Some of the reports discussed will be especially helpful to the general men. Thus when a Wassermann report is returned as "hemolyzed" or "anticomplimentary," etc., the significance of this is explained.

The International Medical Annual†

NINETEEN THIRTY-TWO marks half a century for The International Medical Annual. The 1931 volume is for the forty-ninth year. This follows the general arrangement of previous volumes with subject matter arranged alphabetically throughout. This greatly facilitates ease in finding reviews of progress for the year. There is a cross index at the end of the volume covering sub-topics.

While no review of progress in an annual ever makes any pretense of being altogether complete and inclusive, there is no doubt that few if any volumes are available which cover as thoroughly all phases of medicine.

The reviews are truly international in their scope but, naturally, there is a little predominance of British contributions. This should increase its value to Americans who may not have available all of the British medical literature.

In addition the editors provide an introductory review of outstanding advances that have been made during the year. In this way there is available an eight-page review of the outstanding advances together with a five hundred and twelve page review of outstanding articles on the different subjects.

The illustrations are abundant and excellent.

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*Medical and Surgical Year-Book. Physicians' Hospital of Plattsburgh. Comprising Wednesday Afternoon Invitation Lecturers' Papers of the Cardiac Round Table, The First Beaumont Lecture, Collected Papers by the Staff. Cloth. Pages 322. The William H. Miner Foundation, Plattsburgh, N. Y., 1930.

**Clinical Interpretation of Laboratory Reports. By Albert S. Welch, A.B., M.D., Clinical Instructor in Medicine in the University of Kansas School of Medicine in Kansas City, Kansas; Director of the Laboratory of the Alfred Benjamin Dispensary, and Attending Surgeon of St. Joseph's Hospital in Kansas City, Missouri. Formerly Pathologist to the Kansas City General Hospital, and Chemist to the Coroner of Jackson County. With Sixteen Illustrations and a Frontispiece in Color. Cloth. Pages 366. P. Blakiston's Son & Company, Philadelphia, 1932.

†The International Medical Annual. A Year-Book of Treatment and Practitioner's Index. Editors: Carey F. Coombs, M.D., F.R.C.P., and A. Rendle Short, M.D., B.S., B. Many Contributors. Forty-Ninth Year. Cloth. Pages 551. William Wood & Company, New York.

Arterial Hypertension*

THIS is a very readable review of our present understanding of hypertension into which the author has incorporated his own studies and a very adequate discussion of therapy in general and in specific.

Dr. Stieglitz emphasizes that hypertension is a disease of multiple etiology in which the causes may be roughly divided into those which initiate the progress of hypertension and those factors which tend to perpetuate the hypertension once it has been initiated. He groups the etiologic factors as infectious, intoxication (exogenous and endogenous) and hereditary. The hereditary factors consist in lessened resistance to fatigue of vascular structures, emotional temperament, and obesity.

The diagnosis of the etiology of one case of hypertension practically never resolves itself into a single etiologic factor. The author presents several illustrative cases bringing out this point. For example in one case, the etiologic factors were found to be (1) oral sepsis, (2) excesses in salt, (3) obesity, (4) heredity, (5) anemia, (6) nephritis. In another case the etiologic factors were (1) heredity (emotionalism), (2) pregnancy intoxication, (3) mild hyperthyroidism, (4) recent anxiety. In still another the etiology was (1) oral sepsis, (2) luetic infection, (3) plumbism, (4) alcoholism. Without an adequate appreciation that all of these factors are interacting either as initiating causes or perpetuating causes, the results of treatment will not be optimal.

The pathogenesis of hypertension is discussed in detail and very well illustrated graphically in a panel diagram which displays how irritation of arterial musculature produces muscular spasticity and increased muscle tone which in turn causes hypertrophy of the arterial muscle. Here we run into a small eddy of a vicious circle in which spasticity produces muscular fatigue which in turn results in muscular hyperirritability which produces more spasticity. The two latter factors, hypertrophy and fatigue, are responsible for muscular exhaustion and degeneration which gives place to fibrosis in the replacement of degenerated muscular fibers. This in turn results in the end state, arteriolar sclerosis.

As one might expect, a discussion of the treatment deals principally with the general supervision and only secondarily with drug therapy. The latter however is very adequately covered and a comprehensive presentation is given of the author's own experiences with the use of bismuth subnitrate as a slow but continuous vasodilator. A cursory reader might infer that he recommends the use of digitalis in hypertension more generally than is his actual intention.

The monograph can be highly recommended.

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*Arterial Hypertension. By Edward J. Stieglitz, M.S., M.D., Assistant Clinical Professor of Medicine, Rush Medical College, University of Chicago; Attending Internist, Chicago Lying-in Hospital, Assistant Attending Physician, Presbyterian Hospital. Foreword by Rollin T. Woodyatt, M.D. Clinical Professor of Medicine, Rush Medical College, University of Chicago, Chairman Department of Medicine, Attending Internist, Presbyterian Hospital. With 21 Illustrations. Cloth. Pages 280. Paul B. Hoeber, New York, 1930.

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EDITORIAL

Fungous Diseases

OLD acquaintances, looked upon as of little importance, sometimes step into more prominent rôles. This appears to be the case at the present time with regard to the pathogenic fungi. Curiously enough, fungi were recognized as causes of disease even before Pasteur laid the foundation of modern bacteriology. In 1677 Hooke found that the yellow spots which grow on rose leaves consisted of fungi whose presence he attributed to spontaneous generation. Langenbeck discovered the parasitic fungus responsible for thrush in 1839. In the same year Schoenlein pronounced the fungus origin of favus. The etiology of tinea circinata was announced in 1884, that of pityriasis versicolor in 1846. For years therefore, dermatologists have been interested in the activities of parasitic molds in the skin and on the mucous membranes. Occasionally, members of this group of plant life have been known or suspected as causing internal disturbances such

as actinomycosis, sprue, etc. Recent studies in these and other fields have however given greater prominence to the molds as possible etiologic agents of disease.

Molds are members of the vegetable kingdom, more highly developed than bacteria, but differing from the higher plants in that they do not become differentiated into roots, stems and leaves. They differ from the algae in that in the absence of chlorophyl they cannot manufacture their food from the carbon dioxide of the air but must live off of previously manufactured organic matter. Like bacteria, fungi may be parasitic, saprophytic or facultative parasitic. There are a great number of varieties and species.

Jacobson, whose primary interest is from the dermatologic viewpoint and who has recently published a monograph on *Fungous Diseases* classifies these as (a) primary cutaneous mycoses with usually no definite systemic involvement; (b) primary cutaneous or mucous membrane infections, or both, with frequent systemic involvement; and (c) primary systemic infections with occasional instances of skin or mucous membrane involvement.

In the first group, those limited to the skin, there are two subdivisions, which he designates saprophytic and parasitic. The former is essentially an invasion of the upper layers of the cutis, never becoming deep and is represented by such common manifestations as tinea versicolor and erythrasma. The second or parasitic group penetrate more deeply but here again there is usually no systemic involvement. This group is represented by the tinea infections of the hairy regions and by the familiar trichophyton group.

The second major classification, with skin or mucous membrane involvement and frequent systemic activity include monilia infections, more commonly known as the infections due to yeast-like organisms and the group of devastating but fortunately infrequent dermatologic or systemic diseases which include madura foot, sporotrichosis, blastomycosis, actinomycosis and coccidioidal granuloma.

Jacobson's third classification, systemic infections with occasional instances of skin or mucous membrane involvement is represented by two types of mold infections, torulosis and aspergillosis. The former shows some predilection toward central nervous system involvement, also pulmonary involvement, while the latter involves the lungs by preference.

Although certain of the cutaneous manifestations of these various mold infections are characteristic, enabling one to reach a diagnosis from inspection only, others require biopsy for accurate etiologic diagnosis. When internal organs are involved (bones, lungs, central nervous system, parenchymatous tissues), the clinical picture as a rule does not suggest itself as that of a mold infection but resembles more or less closely some other type of pathology, particularly, pyogenic infection. Undoubtedly the apparent rarity of many of these diseases is due merely to the fact that, not being suspected, laboratory studies have not been made with this in mind. It would be well when examining material from apparently infectious sources within the body, to always bear in mind the possibility of fungus infection. The constantly increasing number of cases that have been treated often for years as pulmonary tuberculosis which are found eventually on sputum examination to be due to mold infection and which often respond beautifully to administration of iodides bears this out.

The wide distribution of molds in nature, in dust, in the air we breathe, and the convincing evidence presented in the last few years that one may become specifically allergic to molds, also suggests the occasional importance of fungi in the etiology of disease.

REFERENCE

Fungous Diseases: A Clinical and Mycological Text. Harry P. Jacobson, Attending Dermatologist and Member of the Malignancy Board of the Los Angeles County General Hospital, Thomas, Springfield, Ill., 1932, pp. 317.

W. T. V.